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In this Issue

Note: Vintage Atomizer Perfume Bottle.
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The Chemist

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The Chemist

Journal of the American Institute of Chemists

The Chemist (Established in 1923)

Editorial

Chemistry in Life

David Devraj Kumar
Florida Atlantic University

Contributions from chemistry to the area of life sciences are enormous. Chemicals in natural or synthetic forms have been used in health and clinical areas as long as history can be traced, and advancements in humanity's understanding of the chemical nature of making and breaking bonds have contributed to unprecedented developments in clinical chemistry. A section in this issue of *The Chemist* is dedicated to the theme "Clinical Chemistry" edited by Dr. Margot Hall. Dr. Hall was kind enough to solicit manuscripts for this themed section, as well as doing all the hard work associated with the process of editing it. For a summary of the manuscripts in this section, I encourage you to read the guest editorial by Dr. Hall in this issue of the journal.

In addition to the themed section on Clinical Chemistry, there are contributions from others. Bethany Davis describes a thought provoking way to encourage the participation of girls in chemistry through exploring the chemistry of makeup. She was invited to write this piece in *The Chemist* based upon her well-known outreach efforts to promote chemistry among girls in schools and colleges. Encouraging the participation of women in general in the sciences is a national priority. On the other topics, Leah Eller presents a review of the book *Organic Structure Analysis* (second edition), and Kenneth Abate reviews the book *Metal-Polymer Nanocomposites*. Finally, a friend of chemistry, Dr. P. V. Thomas is recognized.

It is impossible to edit a journal without the generous help of colleagues. I would like to thank Dr. Penelope Fritzer of Florida Atlantic University for her valuable feedback on book reviews. Thanks to the reviewers from the journal's review board and those who served as ad hoc reviewers contributing their time reviewing manuscripts for *The Chemist*. Also thanks to Wade Berstler of Florida Atlantic University as ad hoc editorial assistant on various tasks associated with editing *The Chemist* this Fall. Finally, a journal is worth nothing without its readership benefiting from its articles. I hope this volume 87 and issue number two of *The Chemist* is of interest to you.

Thank you.

Guest Editorial

Clinical Chemistry

Margot Hall
University of Southern Mississippi

Clinical Chemistry is a subdivision of Biochemistry, which addresses the analytical testing of human body fluids in health and disease. The results of clinical chemistry tests can be used to assist the physician in making a diagnosis, monitoring a therapeutic regimen, determining a prognosis, and even (in some cases) as evidence in forensic cases.

In this issue, Adam Shirley and co-authors report on the making of a fluorescent assay to measure the concentration of Immunoglobulin E in human body fluids. They offer their insights on how this assay might be incorporated into a working hospital clinical lab. In another article, Tammy Shaw and co-authors review the hormones that regulate glucose metabolism and a variety of pathologies, which affect the blood glucose levels. An algorithm for diagnosing these pathologies is given.

In still another article, Erika Harmon Pratte and her co-authors describe the biochemistry and mechanisms of toxicity for four poisons that were popularly used to assassinate one's enemies during the 16^{th}-18^{th} centuries. These included toxins from Curare, Hemlock, and the Castor bean, to the favorite poison of the de' Medici era: strychnine. In a fourth article, Hannah Rice and her co-authors review oncogenes and tumor suppressor genes (anti-oncogenes) and the role they play in the development of benign and malignant tumors.

Thank you.

The Chemist
Journal of the American Institute of Chemists

Quantitation of Immunoglobulin E Using Fluorescence Assay

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Abstract: The following paper describes an experiment to fluorescently tag immunoglobulin E (IgE) with Alexa Fluor® 488 Dye carboxylic acid, tetrafluorophenyl (TFP) ester and measure known concentrations of the dye-protein complex using a Biotek Synergy 2 Micro-plate reader to determine its fluorescence intensity. IgE is a human antibody that mediates immediate hypersensitivity reactions and aids the immune system in defending the body against parasitic infection. Fluorescence is the emission of light from a substance that has been exposed to electromagnetic radiation. Electrons in this substance emit light when they return to their ground state following excitement via electromagnetic bombardment. The characteristics and functions of IgE and fluorescence are discussed followed by a detailed protocol on the tagging of Alexa Fluor® 488 Dye to IgE. The fluorescence is successfully measured using the micro-plate reader on samples with known concentrations ranging between 20 µg/dL to 100 µg/dL using a Gen5 computer software program. Lastly, the author explores how the experiment could be implemented into the clinical setting along with how the procedure could be expanded using fluorescence polarization.

Key Words: Immunoglobulin E (IgE), Fluorescence Assay

INTRODUCTION TO IMMUNOGLOBULIN E

The antibody known as immunoglobulin E (IgE) is responsible for the mediation of immediate hypersensitivity reactions that cause hay fever, asthma, hives, and anaphylactic shock, and for assisting the body's host defenses against parasitic infection. The first biological assay to test for IgE was conducted by K. Prausnitz and H. Kustner in 1921 and is now known as the P-K reaction. They discovered the presence of an unknown serum component responsible for allergic reactions by injecting serum from an allergic patient into the skin of a patient who was non-allergic. They then injected an allergy antigen into the same site and noted the appearance of redness and swelling [1].

IgE was officially identified in 1966 by K. and T. Ishizaka. They immunized rabbits with serum from an allergic patient to make an anti-isotype antiserum. IgG, IgA, IgM, and IgD were then allowed to react to the rabbit antiserum causing their antibodies to precipitate. An unknown anti-isotype antibody still remained in the antiserum after this because it was able to completely block the P-K reaction. This unknown antibody was named IgE after the E antigen found in ragweed pollen [1].

IgE FUNCTION

IgE is the least common immunoglobulin in the body, but can bind with high affinity to Fc receptors on the surface of basophils and mast cells. It has the ability to bind with far greater effectiveness to these high affinity receptors than other immunoglobulin classes. The molecule also possesses low affinity receptors capable of cross-linking to antigens and initiating an immune response. Once IgE binds to an antigen, it initiates conformational changes in the mast cells that cause them to release histamine, serotonin, leukotrienes, and other chemicals responsible for hypersensitivity reactions. Even though IgE is usually associated with allergic reactions, its main function appears to be protection of the body from parasites. Once a parasite has been coated with IgE, eosinophils are alerted, bind to the Fc receptors on the IgE-parasite complex, and destroy the invading organism [2].

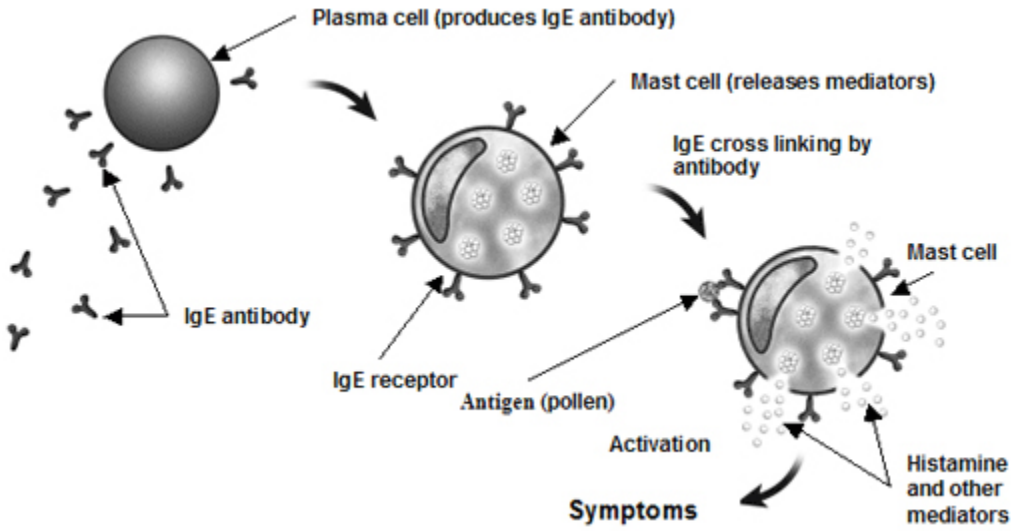

Fig 1. Allergic Cascade with Mast Cell, IgE and Mediator Release (How Xolair Works, 2014). ©2014 The Asthma Center. Used under permission. http://asthmacenter.textalk.com/page32342.html

IgE STRUCTURE

The structure of IgE is made up of two pairs of heavy and light chains that are joined by disulfide bridges. The molecule can be separated into the antibody combing site (Fragment antigen binding) and another fragment (constant fragment) by using a digestive enzyme such as papain. Unlike other immunoglobulins, IgE has four constant heavy chain domains, with the second heavy chain acting as the hinge region. The light chains can be categorized into κ and λ chains, with IgE molecules typically having one type of light chain or the other [3].

The IgE antibody binding site can be found on the variable regions of the molecule at the amino terminal end. Although heavy and light chains can both be integrated into the antigen binding site, it is possible for one type of chain to possess all of the antigen binding activity (Holgate & Kemeny, 1995). The regions that are most active in the antibody combining site are those that have the most variability.

These complementary-determining regions have structures best suited to the binding of antigen and are called CDR1, CDR2, and CDR3. Lastly, near the variable sections of the IgE molecule are framework regions that control the overall structure of the combining site. These regions do show some variation with the μ chains containing four framework regions and the λ chains containing five. The IgE constant region is composed of four heavy chain domains known as ε1, ε2, ε3, and ε4. These regions take part in mediating the effector functions of the molecule [3].

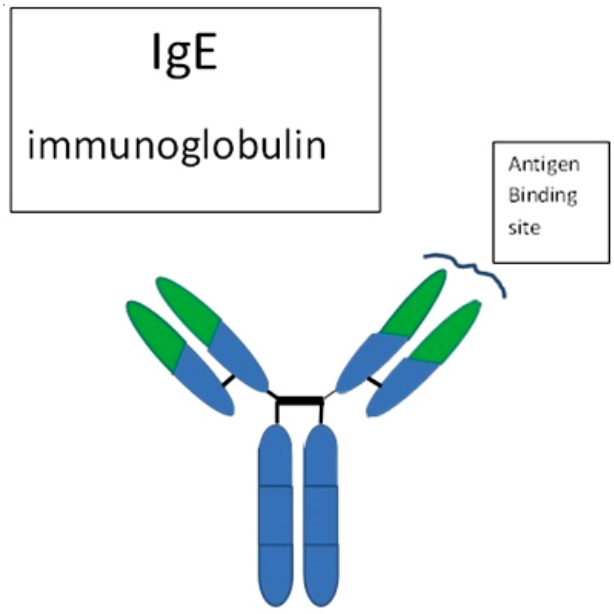

Fig 2. Structure of human immunoglobulin E (Handley, 2014) ©2014 Handley. Used under permission.

IgE QUANTITATION

The normal IgE serum concentration range in adults is 10 – 40 μg/dL [4]. Currently, the most common techniques to detect antigen-specific IgE in serum are radioallergosorbent test (RAST), radioimmunoassay (RIA), and enzyme-linked immunosorbent assay (ELISA). These tests are usually performed on patients suspected to have atopy, Wiskott-Aldrich syndrome, or hyperimmunoglobulin E syndrome. Physicians will also test IgE levels on patients with parasitic infections and bronchopulmonary aspergillosis. IgE is typically not measured in asthma patients due to the fact that serum IgE levels are only elevated in about one half of asthmatics [2].

Introduction to Fluorescence

The use of fluorescence as a diagnostic tool has been of great value to clinical scientists because it allows them to study the structure and function of molecular proteins. Proteins have been demonstrated to emit luminescent light via excitation of ultraviolet light through the experiments of men such as Max Weber, Edwin Teale, and Sergei Konev. The experiments that these men conducted led the way to the discovery of properties of proteins such as their rotational freedom, amino acid side chain exposure, and intramolecular distances [5].

One of the single greatest advantages of fluorescence analysis of proteins is the minimal amount of analyte that is required to perform the experiment. Tryptophan and tyrosine residues are the most studied with tryptophan being the scientific favorite due to its sensitivity to its microenvironment and the fact that most proteins possess few tryptophan residues. The lack of plentiful tryptophan residues allows researchers to be able to pinpoint certain areas of the protein being studied. An example of this lies in the tryptophan indole side chain, where its emission spectrum sensitivity to polarity allows fluorescent probes to be able to differentiate between folded and unfolded protein conformations. This differentiation lies in the ability of fluorophores to compete with other molecules that are emitted on the same spectrum [5].

Instead of using nuclear magnetic resonance (NMR) or crystalline procedures to characterize large multi-protein structures, analysis of the energy transfer between the distances of donor fluorophores to an acceptor can competitively characterize the emission process of the protein structure being studied. This instance does not hinder the electronic dipoles of the molecule and allows accurate energy transfer measurements [5].

Fluorescent Probes

In 1871, Adolph Baeyer synthesized what is now the most popular fluorescent probe in the world, fluorescein. Fluorescein has remained so popular because it is inexpensive, unpatented, and compatible with amine and sulfhydryl functional groups. The probe can take on several different prototropic forms. It retains a dianion form when in alkaline conditions; however, if the pH of solution decreases, one will observe a deprotonated carboxylic acid and a protonated hydroxyl group. If the pH decreases even more the probe will take on a zwitterion form and will become non-fluorescent. This pH dependent characteristic of fluorescein makes its absorption and emission maxima approximately 490 nm and 520 nm at an alkaline pH [6].

Fluorescein isothiocyanate (FITC) is currently the most common form of fluorescein. It was synthesized as a replacement for the isocyanate derivative of FITC and acts on primary amines and lysine residues of proteins. Other forms of fluorescein include iodoacetamidofluorescein, which acts on cysteine residues of proteins, and N-hydroxysuccinimidyl fluorescein, which acts on primary amines of proteins [6].

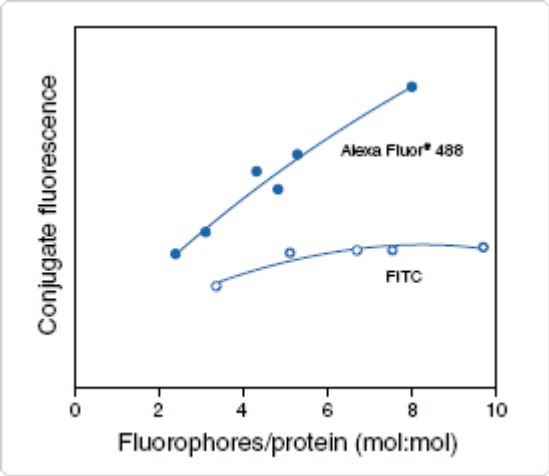

Fig 3. Fluorescence intensity of Alexa Fluor® 488 Dye vs. FITC (Invitrogen, 2011). ©2014 Thermo Fisher Scientific Inc. Used under permission. http://www.b2b.invitrogen.com/site/us/en/home/brands/Molecular-Probes/Key-Molecular-Probes-Products/alexa-fluor/Alexa-Fluor-488-Secondary-Antibodies.html

In the following experiment, the fluorescein fluorophore used was Alexa Fluor® 488 Dye carboxylic acid, tetrafluorophenyl (TFP) ester purchased from Invitrogen. Alexa Fluor® 488 is a green-fluorescent dye conjugate similar to FITC that was introduced in 1997. It produces a brighter conjugate than fluorescein, is less pH dependent, and is more photostable, while still being compatible with FITC filters, making it an ideal fluorophore for this experiment. The TFP ester form acts on primary amines and is now preferred over the original succinimidyl ester form because of its resistance to spontaneous hydrolysis during conjugation and its greater stability at an alkaline [7].

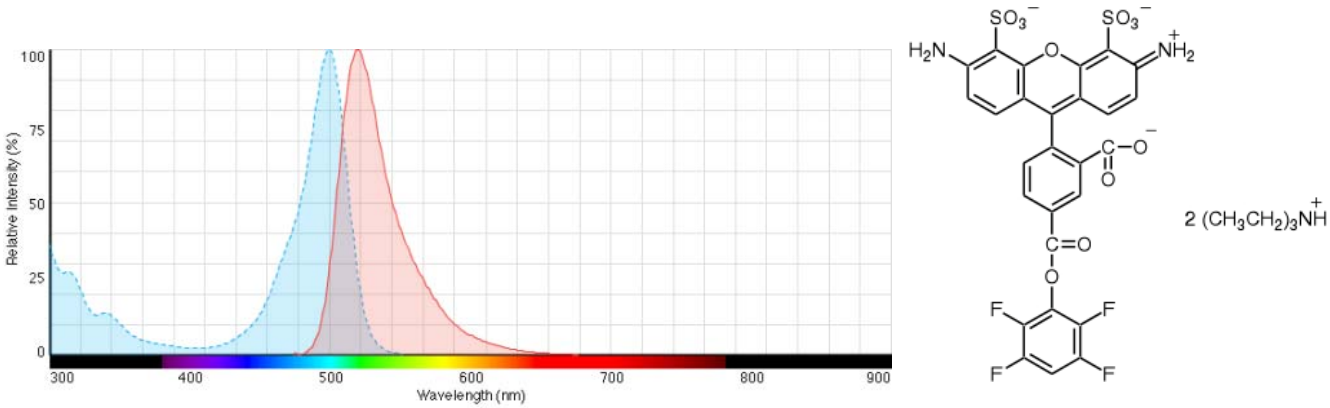

Fig 4. Absorbance/emission spectra and chemical structure of Alexa Fluor® 488 Dye (Invitrogen, 2011).
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http://www.lifetechnologies.com/order/catalog/product/A20181

MATERIALS & METHODS

Tagging of Alexa Fluor® 488 Dye to Human Purified IgE

The tagging of Alexa Fluor® 488 Dye to human purified Immunoglobulin E (IgE) was done using a modified version of the Alexa Fluor® labeling protocol supplied by Invitrogen. Human purified IgE (Immunology Consultants Laboratory, Inc.) at a concentration of 1 mg/mL was added to 1 X Phosphate Buffered Saline (PBS) to make a 0.2 mg/mL concentration solution. 1 M sodium bicarbonate was added to the solution to raise the pH of the mixture and allow succinimidyl esters to react more efficiently. The mixture was then added to the tube of Alexa Fluor® 488 Dye carboxylic acid, tetrafluorophenyl (TFP) ester (Invitrogen) and mixed on a stir plate at room temperature for one hour. Excess Alexa Fluor tag was then removed by centrifugation using Millipore Centrifugal Filter Units (Fischer) leaving a final volume of 500 uL of tagged protein in 1 X PBS and stored at 4°C in the dark.

The concentration of the tagged IgE was determined using a NanoDrop 1000 spectrophotometer (Thermo Scientific). The absorbance of the tagged IgE was measured at 280 nm and 485 nm and the final concentration calculated to be 120 µg/dL using the following formula provided by Invitrogen:

$$\text{Protein concentration (M)} = \frac{[A280 - (A494 \times 0.11)] \times \text{dilution factor}}{\text{molar extinction coefficient of IgE}}$$

where 0.11 is a correction factor to account for the absorption of the dye at 280 nm.

Fluorescence Detection

Several different concentrations of IgE tagged with Alexa Fluor® 488 were diluted in pre-bleed rabbit serum and dispensed into different wells on a Costar® Black 96-well plate (Biotek). Each well had a total volume of 25 µL with one well containing only rabbit serum that was assigned as a blank. The plate was then placed on a rocker and incubated for one hour at room temperature.

The machine that was used to read the fluorescence intensity was a Biotek® Synergy 2 Multi-Mode Micro-plate Reader that utilized Gen5 computer software to organize, interpret, analyze, and store the data that was acquired from the experiment. To run the micro-plate reader, a computer program was designed and written to outline the parameters of what the machine would measure, how it would measure it, and what data would be collected and analyzed. The tagged IgE was measured at a sensitivity of 35 with an 18 mm offset. The offset was the distance between the actual micro-plate reader and the sample well. The data was then interpreted on graphs using Graphpad Prism software.

RESULT & DISCUSSION

The fluorescence intensity results along with the standard curve obtained from the experiment are shown below. The graph plots the fluorescence intensity (FU's) versus the concentrations of IgE tagged with Alexa Fluor® 488 (μg/dL). The concentration of tagged protein in each well was carefully calculated and pipetted so that a standard curve could be obtained that could be compared to the fluorescence intensity measured by the multi-plate reader.

Table 1: Calculated dilutions and concentrations in each Costar plate well.

Entered Values			Calculated Values	
Dilute Solution Conc. (μg/dL)	**Dilute Solution Vol. (μL)**	**Concentrated Solution Conc. (μg/dL)**	**Concentrated Solution Vol. (μL)**	**Serum Vol. (μL)**
20	25	120	4.2	20.8
30	25	120	6.3	18.7
40	25	120	8.3	16.7
50	25	120	10.4	14.6
60	25	120	12.5	12.5
70	25	120	14.6	10.4
80	25	120	16.7	8.3
90	25	120	18.8	6.2
100	25	120	20.8	4.2

As shown on the graph below, a standard curve was created between 20 μg/dL and 100 μg/dL. The normal IgE serum concentration range in adults is 10 – 40 μg/dL [4] and was measured to have a fluorescence of approximately 1500 FU's. A lower concentration of IgE was measured at 20 μg/dL and had a fluorescence of approximately 600 FU's while the higher concentration of 100 μg/dL had a fluorescence of approximately 4000 FU's.

The consistent trend in fluorescence versus IgE concentration shows that human immunoglobulin E can be quantitated using IgE that is tagged with Alexa Fluor® 488 followed by measurement of its fluorescence using a micro-plate reader. Using this method as a clinical diagnostic tool, however, does pose many challenges. The first being that Alexa Fluor® 488 is an extremely expensive fluorophore and would not be cost effective to use on a daily basis. A solution to this problem would lie in the ability of a biotechnology company to standardize this procedure, making it cheaper to produce and thus cheaper to use in the clinical setting. This study had limited resources thus the testing in triplicate and cross-reactivity studies were not performed. This additional research should be performed in the future.

Table 2: Fluorescence measurements obtained for each IgE concentration.

IgE Concentration (µg/dL)	Fluorescence (FU's)
20	621
30	907
40	1300
50	1536
60	2208
70	2385
80	3118
90	3150
100	4071

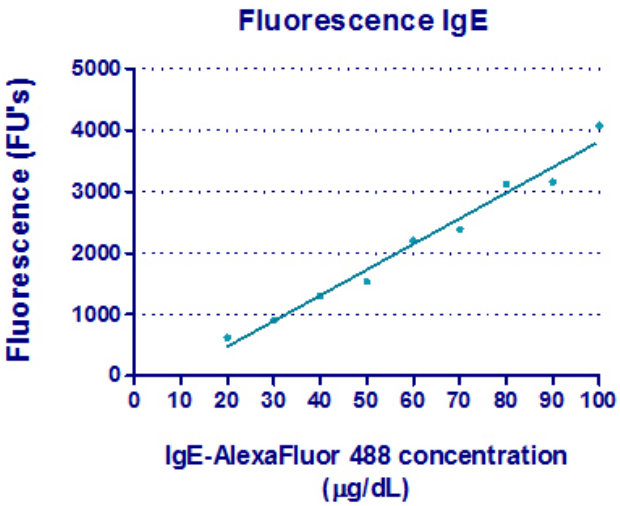

Fig 5. Standard curve showing IgE- Alexa Fluor® 488 Dye vs. fluorescence intensity. ©2011 Adam Shirley original data used by permission

The second hurdle is the time it takes to purify and label the immunoglobulin E from the patient's serum. To solve this issue, the experiment could have been taken a step further and used fluorescence polarization and an antibody to human IgE that was tagged with Alexa Fluor® 488. This would eliminate the need to purify the human IgE and would allow clinical scientists to simply spin down the patient's whole blood and extract their serum. The IgE antibody tagged with Alexa Fluor® 488 would then be added to the serum and the fluorescence polarization would then be measured using the same micro-plate reader and a slightly different Gen5 computer program. This procedure would also be very expensive and would require the need of standardization from a major biotech company to make it cost effective. This additional fluorescence polarization experiment was not performed due the additional expense of the immunoglobulin E antibody but could be performed in the future provided that funding for the experiment was available.

ACKNOWLEDGEMENTS

The author would like to express his sincerest gratitude to Dr. Gordon Cannon and Dr. Sabine Heinhorst of the University of Southern Mississippi Department of Chemistry and Biochemistry for generously donating the Alexa Fluor® 488 and use of their laboratory and Biotek Synergy 2 Micro-plate reader. The author would also like to give thanks to Medical Technology Department at the University of Southern Mississippi; especially Dr. Jane Hudson, Dr. Margot Hall, and Dr. Mary Lux for funding the purchase of the human purified immunoglobulin E. The authors would also like to acknowledge W. Adam Shirley for providing original data from the study in Tables 1 & 2 and Figure 5. Special thanks are also conveyed to Martha Sparrow, Evan Roberts, and Jenifer Milam for sharing their invaluable knowledge of writing, math, and science. Lastly, the author would like to express his love and gratitude to his wife Megan for her understanding and support throughout the course of this project and for being his inspiration in life.

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The Hormones of Glucose Metabolism

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Abstract: Like every other process that is crucial to homeostasis, glucose metabolism is regulated by the endocrine system. There are many hormones that impact this metabolism indirectly due to their roles in key processes such as circulation, appetite, or stress response; however, this paper will examine the hormones that directly regulate or impact glucose metabolism. Additionally, an overview of glycemic disorders is provided.

Key Words: Glucose metabolism, endocrine glucose regulators, endocrinopathies of glucose metabolism.

GLUCOSE

Glucose is a simple carbohydrate that is the human body's primary source of energy [1]. Circulating glucose is in the monosaccharide form, but is stored in the polysaccharide form as glycogen. Although the body can use other sources, such as fats and proteins, to produce energy, these alternative energy sources cannot substitute for glucose in red blood cells or brain cells [2]. Glycogen, however, has recently been proven to serve as a glucose equivalent within the brain, acting as a reserve energy source in the event of hypoglycemic states [3].

To understand glucose metabolism, it is important to first recognize that it is not a singular process, nor is there a common starting point. In fact, it is continuous activity with multiple sources, mechanisms, locations, and triggers [4]. There are four pathways of glucose metabolism: glycolysis, gluconeogenesis, glycogenesis, and glycogenolysis. By understanding each of these processes, one can get a better picture of the roles of each pertinent hormone and their relationship to one another.

GLYCOLYSIS

The process by which glucose is broken down into usable energy forms is called glycolysis. Within cellular cytoplasm, each glucose molecule is cleaved and then converted into pyruvate, creating adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NADH) by the glycolysis reactions. This is a lengthy process involving several enzymes, but regulation of the glycolytic flux depends on the three enzymes that catalyze the irreversible steps: hexokinase, phosphofructokinase, and pyruvate kinase [5]. The relationship is direct—glycolytic activity increases and decreases in relation to the fluctuations in these enzyme levels.

GLUCONEOGENESIS

Gluconeogenesis is the synthesis of glucose from non-carbohydrate forms. The primary location for this synthesis is typically the liver, with minimal synthesis occurring in the kidneys [6]. The enzymatic reactions of gluconeogenesis are mostly the reverse of glycolytic steps, with substitutions of glucose-6-phosphatase, fructose-1,6-biphosphatase, and phosphoenolpyruvate carboxykinase for (respectively) hexokinase, phosphofructokinase, and pyruvate kinase [7]. These mechanisms inhibit one another, thus preventing them from reacting simultaneously and forming a futile cycle [8].

GLYCOGENESIS

When glucose is converted to its storable form glycogen, this process is called glycogenesis. In a properly

functioning metabolic system, glycogenesis occurs in the liver and muscle cells in response to excess circulating glucose without increased energy demand [9]. Glycogen then lies in storage until glucose levels fall, or there is an increased demand for glucose that is not otherwise being met within the body. Once this need arises, the process of glycogenolysis takes place.

GLYCOGENOLYSIS

In the liver and muscle cells, stored glycogen is broken down into glucose-1-phosphate. Skeletal muscle is technically capable of generating glucose from glucose-1-phosphate, which could then enter the bloodstream. However, the abundance of hexokinase in these cells immediately phosphorylates any free glucose before it can do so and it enters the glycolytic pathway instead. In the liver, glucose-1-phosphate is further converted to glucose-6-phosphate by phosphoglucomutase [10]. This is then either distributed for use in energy production, or further converted into glucose for dispersion to the tissues unable to utilize the phosphorylated form.

HORMONAL REGULATORS OF GLUCOSE METABOLISM

The previously described metabolic pathways are regulated and/or influenced by several hormones. Epinephrine, cortisol, growth hormone, thyroid hormones, somatostatin, and ACTH are all implicated in glucose metabolism. They express their effects on metabolic pathways directly by affecting components or steps of the pathway, indirectly through influences on insulin and/or glucagon, or a combination of these routes [11]. However, the primary hormones responsible for maintaining glucose homeostasis are the pancreatic hormones insulin and glucagon.

INSULIN

A peptide hormone synthesized from proinsulin by the pancreas, insulin is the most widely recognized glucose-mediating hormone. It is secreted continuously to maintain circulating glucose levels, and secreted in increased amounts in response to exogenous stimuli [12]. When the β-cells of the islets of Langerhans detect elevated circulating glucose, they secrete insulin. This response appears as though secretion is happening purposely in two separate bursts; however, recent research indicates that the immediate peak is caused by the rapid release of stored insulin. The subsequent drop and secondary peak is caused by the depletion of stores followed by the secretion of newly created insulin [13].

There are several mechanisms by which insulin produces its glucose-lowering effects, and these actions take place in several different organs and tissues. In the liver, insulin stimulates glycogen synthesis by activating hexokinase. This promotes storage of glucose (as glycogen), thus lowering circulating glucose levels. It also inhibits the ketogenesis and gluconeogenesis by disrupting enzymes involved in each process [12]. By inhibiting gluconeogenesis, circulating glucose levels are prevented from increasing.

Adipose tissue—the fatty tissue—is another area that involves significant levels of insulin activity. Adipose tissue is comprised of triglycerides, and it is these triglycerides that are the object of the insulin effects [14]. Insulin prompts lipoprotein lipase production, which in turn hydrolyzes triglycerides from circulating lipoproteins allowing the fatty acids to enter adipose cells. Additionally, insulin enhances α-glycerol phosphate availability, which is used to esterify free fatty acids into triglycerides inside the adipose cells. Lipolysis of stored triglycerides requires lipase whose intracellular production is inhibited by insulin. The result of all of these mechanisms is increased triglyceride storage and reduced circulating fats.

Insulin activity within muscle tissue is more diverse than that of adipose tissue, primarily due to the complexity of muscle composition and action. As in the liver, insulin promotes glycogen synthesis. In the muscle, this is achieved as a result of three insulin actions: glycogen phosphorylase inhibition, glycogen synthase enhancement, and increased transport of glucose. Intracellular glucose transporters are otherwise inactive until insulin binds to receptors [15].

There are multiple glucose transporter proteins (i.e. GLUT 1 through GLUT 4) with different affinities for glucose and a time lag following binding insulin to the receptor during which the transporter protein translocates to the cell membrane. The difference in the affinities allows preferential release of glucose to brain and muscle tissue (i.e. GLUT 2 has low affinity for glucose and this causes decreased uptake and storage by hepatic cells during fasting. Another example would be when GLUT 4 is sequestered in intracellular compartments of cells and is not able to function as a transporter until it receives the signal from insulin binding to its cell membrane receptor, at which time GLUT 4 translocates to the cell membrane

[12]. Insulin also increases protein synthesis within the muscle tissue. This effect is due to insulin's combined increases in ribosomal proteins production and amino acid transportation.

GLUCAGON

Glucagon, like insulin, is a peptide hormone synthesized in the pancreas. It is also responsible for glucose homeostasis, but its role is the exact opposite of insulin. Secreted by the α-islets in response to low circulating glucose, glucagon prompts several actions that result in an increase in circulating glucose [16]. Glucagon secretion is also stimulated by various amino acids and catecholamines independent of glucose levels. It is primarily inhibited by glucose, but insulin and somatostatin's inhibition of α-islet cells also reduces glucagon secretion [17].

Like insulin, glucagon's actions take place in a variety of organs and tissues. The liver holds the largest concentrations of glucagon receptors, which results in the majority of glucagon activity impacting hepatic mechanisms. Glucagon binds to adenylyl cyclase receptors, inducing gluconeogenesis [18]. When amino acid precursors are depleted, glucagon stimulates enzymatic break down of stored glycogen [19]. When glycogen stores are depleted, ketogenesis is prompted in the form of acetoacetate, β-hydroxybutyrate, and acetone [20].

Glucagon receptors also exist in adipose tissue, kidneys, the heart, pancreas, gastrointestinal tract, thyroid, and central nervous system [21]. In the kidneys, glucagon stimulates cyclic adenosine monophosphate production [22]. Additionally, glucagon increases amino acid concentration of glomerular tissue, inducing glomerular enlargement. As a result of these two actions, vasodilation and increased glomerular filtration rates occur [23], prompting a decrease in blood pressure.

In adipose tissue, glucagon increases lipolysis [24] by triggering the release of fatty acids from triglycerides. In the gastrointestinal tract, glucagon produces an anti-spasmolytic effect [25], and relaxes smooth muscle of the upper and lower tracts [26]. Glucagon increases both the rate and force of cardiac contractions [27], as a result of glucagon's stimulation of cyclic adenosine monophosphate production and calcium channel activity.

PATHOLOGIES OF ENDOCRINE IRREGULARITIES

Abnormalities of the aforementioned hormones, either in function or amount, will result in imbalances of circulating and/or stored glucose. These glucose homeostasis disorders can be divided into three classifications: hyperglycemia, hypoglycemia, or congenital defect of metabolism. Humans require constant sources of energy, which is primarily derived by the metabolism of glucose and disruption of this metabolism can result in severe and devastating effects.

HYPERGLYCEMIC DISORDERS

Chronic hyperglycemia is most commonly caused by diabetes mellitus, which is a syndrome of deficient or ineffective insulin. This disorder is divided into two classifications (type 1 and type 2), and is further defined by dependence on insulin intervention. Diagnosis is prompted after investigation of the etiology for persistently elevated serum glucose levels. Patient symptoms usually result in the medical practitioner's order of blood glucose, insulin and ketone testing. However, Type 2 may remain asymptomatic for several years [28], with diagnosis resulting from the detection of hyperglycemia during routine laboratory testing.

Type 1 diabetes mellitus.

Type 1 diabetes mellitus is the more severe of the two diabetes types, causing critical long- and short-term complications. It is characterized by the failure of the pancreatic beta cells to secrete insulin in response to circulating glucose. Prognosis improves the earlier it is detected and treated. Long-term complications to vascular, retinal, renal, and immune system function are related to the success and consistency of glycemic control [29]. Left untreated, the resulting production and accumulation of ketones is inevitably fatal. Treatment involves a combination of diet (low sugar) and insulin injections.

Pancreatic dysfunction of type 1 diabetes has several idiopathic and immune-mediated origins, but the most prevalent cause is genetic mutation [30]. Several genes have been identified that contribute to its onset, with various confounding factors increasing risk. The mutations implicated in diabetes disturb immune system

function, resulting in autoimmune destruction of pancreatic β-cells [31]. Mutations of insulin receptors also are rarely implicated. The implicated mutations are not a guarantee that type 1 diabetes will develop, but isolating the specific gene mutation (or combination of gene mutations) helps determine the susceptibility. As such, genetic mapping of children with familial history of type 1 diabetes can serve as an essential early intervention strategy [32]. By identifying the potential for type 1 diabetes development early, immunosuppressant therapy can prevent autoimmune destruction of the pancreatic cells.

Type 2 diabetes mellitus.

As with type 1 diabetes, type 2 diabetes mellitus is characterized by chronic and persistent hyperglycemic states. Elevated glucose levels are caused by insulin resistance, as opposed to insulin deficiency [12]. Several mechanisms associated with insulin resistance have been identified, but the underlying cause of these mechanisms remains undefined. Risk factors include obesity, diet, gender, and race; additionally, several gene mutations increasing susceptibility have been identified [33].

Pharmacological management of type 2 diabetes involves medications that lower blood glucose. Medications that stimulate insulin production and secretion, or inhibit enzymatic carbohydrate metabolism may be added if insulin sensitizers are inadequate [34]. Insulin injection is used in cases where hypoglycemic oral agents fail, but combined therapies in early disease management have gained popularity [35] due to insulin's protective effects on β-cells [36].

Modification of diet and exercise can reverse insulin resistance in many type 2 diabetics, especially in obese patients [37]. In pre-diabetic patients, diet and exercise can also prevent development of type 2 diabetes [38]. Non-obese type 2 diabetics, who compose up to 40% of the type 2 population, are also typically responsive to diet and exercise. However, exercise-induced insulin improvements of non-obese patients are strictly short-term and relative to consistency [39] whereas obese patients can see long-term improvements due to decreased body fat.

Diagnosis of diabetes mellitus.

Laboratory findings of fasting glucose levels greater than 126 mg/dL (7.0 mmol/L), diabetic symptoms combined with glucose levels greater than 200 mg/dL (11.1 mmol/L), or 2-hour glucose measurement during a glucose tolerance test (GTT) greater than 200 mg/dL (11.1 mmol/L) are definitive diagnostic criteria. Most type-1 diabetes patients become symptomatic, and are therefore diagnosed, in childhood. However, in the event of uncertainty, presence of islet cell autoantibodies can conclusively differentiate between type 1 and type 2 [40]. Additional laboratory findings of diabetes mellitus also include glucosuria, hyperlipidemia, ketonuria, proteinuria, and microalbuminuria.

HYPOGLYCEMIC DISORDERS

Hypoglycemia can be caused by a wide variety of diseases, disorders, physiological states, and pharmaceutical reactions. True hypoglycemic disorders are differentiated by the absence of an external cause (known as reactive hypoglycemia), and can be broadly classified as hyperinsulinemic or non-hyperinsulinemic [40]. Inadequate glucose causes nausea, vomiting, cognitive impairment, and emotional lability. Left untreated, hypoglycemic patients will progress into unconsciousness, coma, and eventually death.

Hyperinsulinemic hypoglycemia.

Excess insulin has an obvious effect of lowering circulating glucose levels to a hypoglycemic state. Upon presentation of hypoglycemia and hyperinsulinemia, the etiology of the excess insulin must be determined. In the absence of a chronic disease, the sudden occurrence of hypoglycemia in an adult is most commonly due to an insulin secreting tumor of the pancreas [12]. If exogenous causes of hyperinsulinemia have been ruled out, autoantibodies to insulin should be considered.

Several drugs can induce hypoglycemia, and the sudden onset of hypoglycemia in an otherwise healthy adult is most commonly drug-related [41]. Patients presenting with a seemingly spontaneous hypoglycemic event warrant a complete investigation of prescription and over-the-counter medications. Sulfonylurea drugs are one of the most commonly implicated, and are especially prevalent in patients with renal insufficiency [42].

Non-hyperinsulinemic hypoglycemia.

The cause of hypoglycemia without a corresponding elevation in insulin requires an extensive examination of clinical presentation and laboratory findings, as there are

numerous possible causes. Dietary and pharmaceutical intake, environmental factors, extra-pancreatic endocrine disorders, renal or liver disease, and carbohydrate or fatty acid metabolism disorders can all induce hypoglycemia [43]. Traumatic injury and severe illness, primarily of the liver, kidneys, or pancreas can also cause hypoglycemia. Symptom severity varies relative to glucose levels, but gradual and chronic onset may result in lack of patient awareness of the symptoms [44].

As mentioned earlier, the liver plays a vital role in glucose homeostasis. Understanding this, the dysfunction or inhibition of the liver is the mechanism of hypoglycemia in many etiologies. Ethanol and salicylates are hepatotoxic, resulting in inhibited gluconeogenesis [45]. Diseases of the liver and kidneys also inhibit gluconeogenesis, resulting in lowered circulating glucose. Extra-pancreatic endocrine disorders that induce hypoglycemia are those that result in deficiencies of growth hormone, adrenal insufficiency, hypopituitarism, and thyroid dysfunction. The presence of symptoms that can't be explained by hypoglycemia (such as stunted growth) help identify the appropriate etiology.

Carbohydrate and amino acid metabolism disorders (also called inborn errors of metabolism) are predominantly found in newborns and infants. Glycogen storage disorders, caused by defects or deficiencies in enzymes of the glycogenesis pathway are characterized by suppressed insulin secretion and elevated glucagon levels [40]. Galactosemia and fructose intolerance are caused by enzyme irregularities that inhibit the breakdown of, respectively, galactose and fructose. Glucose synthesis is diminished or absent, and accumulations of galactitol in tissues results in irreversible optic, hepatic, and neurologic damage [46].

Diagnosis of hypoglycemic disorders.

Unlike the hyperglycemic states, the diagnosis of hypoglycemic disorders is not dependent on the presentation of hypoglycemic symptoms. Neonatal screening for inborn errors of metabolism as a result of an increased understanding of their importance has resulted in earlier diagnosis – often before clinical symptoms even appear [47]. The more thorough investigations of neonates with family history of metabolic disease have improved early diagnosis rates [48]. In the absence of a previously identified metabolic disorder, fasting evaluations are performed. Patients are required to fast and serial blood glucose testing is performed at specified intervals. The urine output also is tested for ketones during the fasting period. Upon presentation of either hypoglycemic symptoms or glucose levels below 45 mg/dL (2.5 mmol/L), insulin levels are tested in conjunction with the glucose measurements. Intravenous glucagon is administered at the termination of the fast, and blood is drawn every 10-15 minutes post-administration for glucose testing [40].

Evaluation of the insulin concentration in relation to the glucose levels at the same time serve as the first step in determining etiology of hypoglycemic states [49]. If insulin levels are elevated, C-peptide levels—using samples from the same timeframe—are then used to differentiate between exogenous and endogenous insulin sources [50, 43]. Once this is determined, more specific tests such as insulin autoantibodies, sulfonylurea, β-hydroxybutyrate, and free fatty acids can be utilized to identify the source of disproportionately elevated insulin. When clinical symptoms are consistent with hyperinsulinemic hypoglycemia without an elevated insulin-to-glucose ratio, care should be taken to exclude the possibility of insulin-like substances, especially in the absence of urine ketones [40].

Diagnostic investigations that follow hypoglycemia *without* corresponding hyperinsulinemia are first distinguished by the presence of ketones (or lack thereof) and free fatty acids. If free fatty acids are elevated but ketones remain normal or are suppressed, disorders of fatty acid metabolism are the likely culprit [51]. When ketones are also elevated, endocrinopathies, liver or renal disease, ingested substances, and inborn carbohydrate and amino acid metabolism disorders should be considered. Laboratory evaluations to rule out or confirm suspected causes will vary, as the patient's symptoms lead to the narrowing of possible etiologies.

Other causes, such as substrate inhibition or increased utilization, may also be the origin of the hypoglycemia. Hypoglycemia caused by sepsis is apparent when evaluating blood counts in connection to hypoglycemia, although such observations are viewed as a marker of disease severity [52]. In some of these causes, the clinical assessment can be more definitive than laboratory findings. For example, severe malnutrition or excessive physical exertion is easily discernible to the practitioner, but may result in a variety of laboratory results that fail to point to an obvious cause [53]. Severe dehydration resulting from gastrointestinal illness may also prompt hypoglycemia with an electrolyte imbalance as the only other demonstrated laboratory abnormality [54].

Treatment for hypoglycemia often involves regulation of the timing, type (carbohydrate, lipid, protein) and volume of food intake.

In conclusion, a knowledge of the hormones involved in glucose regulation and access to clinical testing for these hormones is essential for the correct diagnosis of hyper and hypoglycemic disorders. Additionally, genetic mapping and genetic engineering can lead to early and more successful therapeutic interventions.

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Oncogenes and Tumor Suppressor Genes: An Essential Building Block of Cancer

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Abstract: Cancer is a hyperplastic cellular malignancy that affects approximately 1.5 million Americans annually. Oncogenesis is associated with both genetic predisposition and environmental onslaught, with a mixture of the two being required for the malignancy to progress. An understanding of the underlying genetic injuries is useful in the research of cancer and its prevention and treatment. This paper will present a short literature review of the role of oncogenes and tumor suppressor genes and their role in the initiation of malignancy.

Key Words: Oncogenes, Tumor Suppressor Genes, Cancer, Malignant, Benign.

INTRODUCTION

The disease state known as cancer is aggressive, degenerative and affects many people worldwide. In 2014, it was estimated there would be 587,720 cancer deaths in the United States alone, and 1,665,540 new diagnoses [1]. In 2012, there were 14,100,000 cases diagnosed worldwide in that single year [2]. Birindelli et al. [3] described cancer as "the result of circumvention of the apoptotic machinery, promotion of cell division and cell proliferation, loss of cell differentiation pathways, and disruption of cell-cell communication and interaction". All of these stages can be traced back to the activity of oncogenes.

The beginning of cancer, known as the process of carcinogenesis, is composed of many steps involving specific genes prone to producing such a state and signals provided and controlled by the products thereof [3]. The resulting disease state produces malignant tissue that invades and destroys nearby tissue and can metastasize to other areas of the body [4]. There is a large genetic component to cancer since it arises from alterations in cellular DNA or in the transcriptional or translational processes that produce abnormalities in gene expression [5]. Although all cancer is not hereditary, over 200 hereditary cancer syndromes have been described, and an individual's risk for cancer is increased if multiple family members are afflicted [6].

RATIONALE

Basic knowledge of the genes involved in carcinogenesis is a valuable tool in the investigation of cancer and the potential for a resultant therapeutic solution. Oncogenes and tumor suppressor genes are the primary entities involved in carcinogenesis and thus it becomes necessary to understand them to understand cancer on a molecular level. All cancers have a genetic component, whether somatic or inherited [5]. This statement alone indicates the importance of studying the genes implicated in carcinogenesis, as they may provide some method of predicting its occurrence. The alterations of the genes therein may also allow for more accurate diagnosis of cancer by providing molecular markers of its appearance [3]. The targets of action of the revised gene products are primarily the commitment to DNA synthesis in the G1 phase of the cell cycle and the commitment to mitosis in the G2 phase [7]. This functional alteration contributes greatly to the neoplastic phenotype by allowing a cell to both proliferate freely and escape regulation by apoptosis. Such alterations of proto-oncogenes (genes that code for protein products that regulate cell proliferation) and tumor suppressor genes are caused by many insults such as chemicals, ionizing radiation, and viruses.

DISCUSSION

Oncogenes arise from proto-oncogenes that regulate the cells' signaling pathways. When a mutation occurs in a proto-oncogene that activates it to oncogene status, production of the protein produced by the transcription thereof is either increased or the protein itself is altered in structure or function [6]. Proto-oncogenes normally encode proteins that act to promote cellular proliferation by participating in signaling pathways that relay growth stimulating signals through cells and are essential to many normal cell functions [5]. This communication proceeds by way of a cascade of intracellular biochemical signals and the result is activation or repression of various genes. The function of proto-oncogenes is critical to the control of normal cell growth and differentiation [7]. Growth factors and growth factor receptors are two types of pro-oncogene products that get altered by an oncogene [5]. Proto-oncogenes are often located near chromosomal breakpoints, making them targets for potential mutation and increasing the likelihood of carcinogenesis [7].

Normal cells, in which isolated occurrences of these mutations occur, have several methods of combating their destructive effects. One method of regulation is the traditional cellular technique of altering the controlling enzymatic activity. Transcription factors also regulate cell behavior by affecting growth factor genes, among others. If the pathway to apoptosis remains intact, the cell may activate this "self-destruct" function to eliminate the offending gene or gene product. The complexity of the regulatory processes controlling the expression of proto-oncogenes has two implications for neoplastic (cancerous) cells: 1) the large number of components involved provides a large number of potential mutation targets, and 2) multiple regulatory pathways ensure that mutation/carcinogenesis must occur in multiple proto-oncogenes to be effective [5].

Activating mutations, that is, mutations resulting in the beginning stages of carcinogenesis, are usually somatic events [5]. A somatic event refers to one occurring as the result of some outer stimuli and not from genetic inheritance. Loescher and Whitesell [5] predict that inherited mutations would likely be lethal during embryonic development. Mutations affect normal cellular processes such as apoptosis, signal transduction, and DNA replication [7]. Oncogenes can also be initiated by viruses to start the carcinogenic process. Viruses such as the Hepatitis C virus (HCV), Epstein-Barr Virus (EBV), and Human T-Cell Leukemia Virus-1 (HTLV-1) mimic components of the cellular signaling pathways like ligands and receptors to affect production of oncogenic proteins [8].

Oncogenes are classified into six different categories. One category consists of growth factors involved in cellular communication such as proliferation, growth, differentiation and survival. Their overproduction is associated with cancer and has proven to be a key to the process of angiogenesis. Examples include vascular endothelial factor in breast and colorectal cancers and fibroblast growth factor in hemangiomas. A second category is growth factor receptors. They relay the signals conducted by growth factors to the target cell. Their overproduction is also associated with cancer as they continue to release proliferative signals in the absence of growth factors. Their overexpression is especially found in non-small-cell lung cancer, breast, ovarian, and colorectal cancers. Category three consists of nonreceptor tyrosine kinases that initiate tyrosine kinase activity (phosphorylation of tyrosine residues) in the absence of receptors as their name suggests. They are also found to be increased in cancers such as neuroblastoma and small-cell lung cancer [5].

A fourth category is membrane-associated G proteins, or guanine nucleotide-binding proteins. These proteins act as on-off switches (signal transducers) for growth factor receptors on the cell surface. Their increase is implicated in cancer by altering the cell membrane in malignant transformation as well as transmitting stimulatory signals without prompt. These oncogenes are implicated in two-thirds of malignant tumors, a fraction containing virtually all types of human cancer. The fifth category is serine threonine kinases, which are components of intracellular signal transduction. They begin a cascade leading to cell division and are over-stimulated in cancer states. Transcription factors are the sixth and final category of oncogenes. They bind to DNA and initiate changes in gene expression, either regulatory or transformational. These factors can cause chromosomal translocation resulting in a malignant phenotype and are associated with Ewing's sarcoma, clear-cell sarcoma, alveolar rhabdomyosarcoma, and several kinds of leukemia [5].

One proto-oncogene whose mutation often produces cancerous effects is named c-myc. If c-myc is up-regulated, a transcription factor is produced in excess which results in transformed cells and rapid cell proliferation. The concomitant mutation of bcl-2 with c-myc mutation results in the cells' additional ability to escape apoptosis, producing both an immortalized and a transformed phenotype [9]. Both c-myc and bcl-2 have diagnostic and

prognostic value in suspected cancer patients [3]. Bcl-2 acts as a potent inhibitor of apoptosis and becomes overexpressed by chromosomal rearrangement, especially in lymphoid malignancy. The study of bcl-2 became the origin of the study of apoptosis and cancer. [9].

Yet another example of oncogene mutation is found in the effects of the oncogenic virus, human papilloma virus (HPV). HPV encodes a protein known as E7, which inactivates the RB (retinoblastoma) gene whose function is cell regulation. The result of this inactivation is stimulated cell cycle progression. Viral-induced mutation alone is not enough to induce total transformation, because it usually leads to apoptosis [9]. Since apoptosis ends with the death of the cell, the inhibition of apoptosis is the process that is key to carcinogenesis.

Tumor suppressor genes are those whose protein products negatively regulate cell growth by blocking the action of growth promoting proteins [5]. Some have been seen to directly antagonize the action of proto-oncogenes in growth regulation [10]. Some of these genes are normally active transcription factors within the cell nucleus. Abnormal repression of tumor suppressor genes results in deregulation of the cell cycle (excess cellular proliferation by prolonging proliferation signals) or cellular disorganization [5].

Tumor suppressor genes (TSGs) function in the cell cycle by inducing checkpoints, pauses, or arrests in certain stages of the cycle. These checkpoints allow for DNA repair and act to ensure the integrity of the cell's genome [9]. TSGs mutations differ from other potential carcinogenic mutations because the alteration of TSGs produces a modification in the protein products that directly contributes to the transformed phenotype, as opposed to the other classes (proto-oncogenes, DNA repair genes) which produce indirect effects. More than 12 such genes have been localized and identified, though some of these may be mislabeled due to their change in expression in cancer cells [10].

The majority of hereditary cancers are caused by mutation of tumor suppressor genes in germline cells. TSGs require a germline mutation and a somatic mutation or two somatic mutations to initiate carcinogenesis. This theory is referred to as Knudson's Two-Hit Hypothesis and is based on Knudson's study of the autosomal dominant inheritance witnessed in epidemiological studies of retinoblastoma [6]. The first damaged allele may be transmitted in the germline cells of offspring with the second mutation of somatic origin, or both mutations may be somatic. The first mutation (germ line) is small and confined to one gene, otherwise it would be lethal and the process would end. The second mutation results in complete loss of all or part of a chromosome [5].

Methods for identification have been circuitous at best but studies, have yielded several useful facts. To begin with, inheritance of TSGs seems to be dominant in contrast to other cancer-related genes, but they behave recessively on the cellular level [6]. Additionally, fully functional TSGs may suppress tumorigenicity even in the presence of activated oncogenes, indicating the importance of these genes in normal cell functionality and regulation [10]. Suppression of TSGs is crucial to the progress of carcinogenesis as is evidenced by the observation of Park [7] that oncogene expression precedes tumor formation by several months, with the conclusion that other events must be necessary.

TP53 (the p53 gene) is a tumor suppressor gene which codes for the protein called p53. It is the most commonly mutated gene in human malignancy. Even heterozygous mutation of p53 is associated with high rates of carcinogenesis in many tissues [9]. This gene is normally present, but in very low levels with a short half-life and acts as a transcription factor, activating or repressing the expression of various genes [8]. Assault in the form of DNA damage or viral infection may produce up-regulation of p53 to protect the cell. Loss of p53 as effected by many methods of carcinogenesis correlates with a loss of or resistance to apoptosis. The method of this action is unknown. Mutation resulting in p53 inactivation is unique in that it both eliminates a negative regulatory control on proliferation and increases the cell's resistance to apoptosis. This double utility may indicate an explanation for the frequency of the mutation [9]. Mutations of p53 and its corresponding gene TP53 are also used as prognostic tools indicating poor prognosis, increased risk of relapse, and cancer-related death [3].

Another gene, known as the retinoblastoma gene (RB), is also inactivated in many tumors and represents the first isolation of a tumor suppressor gene [5]. So called because it was first studied in the inheritance of retinoblastoma, RB gene functions as a cell cycle regulator. It interacts with a family of transcription factors to inhibit their activity and prevent entrance into the synthesis (S) phase of the cell cycle [9]. Phosphorylation of RB is required for entrance to this phase [11]. Like p53, mutation of even one RB allele increases the possibility of carcinogenesis in many tissues [9]. Retinoblastoma occurs via Knudson's Two-Hit Hypothesis where a loss of heterozygosity (loss or damage of a functioning allele) increases the likelihood of tumor formation and a second "hit" causes the malignancy [10].

In some cases, and possibly more often in the future, these genetic alterations of proto-oncogenes and tumor suppressor genes may be used as tumor markers for diagnosis of different types of cancer. They may also have some prognostic value to categorize patients based on predicting their likelihood of relapse and response to treatment [12]. For example, bcl-2 is found in prostate and breast cancer (both tumors responsive to hormones) and has prognostic value in the measurement of the level of its expression. The oncogene myc is found to be amplified in breast cancer, neuroblastoma, and retinoblastoma, can be a progression marker in advanced stage cancer, and is also related to poor prognosis in solid tumors [3].

CONCLUSION

In conclusion, evidence abounds for the crucial relationships of oncogenes and tumor suppressor genes with the process of carcinogenesis. Activation of multiple oncogenes and inactivation of several growth suppression genes is required for the acquisition of a completely neoplastic phenotype [7]. This is the result of the incorporation of oncogenes and tumor suppressor genes in signaling pathways and cellular regulation [13]. Oncogenes and tumor suppressor genes can be said to cause cells to ignore warning signals at their built-in checkpoints concerning the cell cycle, such as DNA damage, replication defects, or chromosome attachment to the spindle [7]. The gene alterations thereof can be used not only to better understand the disease state and underlying mechanisms, but also as both diagnostic and prognostic indicators of the disease process in the human body.

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Pick Your Poison

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Abstract: During the 16th to the 18th centuries, the royal courts were renowned for their dangers, intrigues and deceptions. There were constant threats to the throne and there are entire libraries dedicated to the lengths a royal family would undergo in order to ensure that their offspring would either inherit the throne or to ensure they themselves would keep the throne. One way that royalty could ensure that their family line stayed in the ruling position was simple … remove the threat. This time in history is filled with tales of *this* royal having *that* royal poisoned, *this* mistress having *that* wife killed and so forth. The list is endless. Though there were many routes the royals utilized to exterminate their competition, some fairly inventive, this paper will focus on the more mysterious aspect utilized: poisoning. This topic was inspired by certain special ladies of the royal court such as Catherine de' Medici, the Marquise de Brinvillers and Catherine Deshayes, or as she was later called "La Voisine" (Hooper, 2006). This paper will focus on a select few of the plant derived poisons. Some of these poisons need to be introduced into the body via the skin while others can simply be ingested or inhaled. While there are some poisons that simply irritate the skin this paper focuses on poisons with serious enough side effects to end the life of an enemy. The poisons included in this paper are separated into three groups: poisons found in the wild, a poison that can be found in a garden (i.e., can be homegrown) and the last section covers the major poison of the Medici age. In the "Poisons Found in the Wild" section the toxins from the Curare plants and Hemlock plants will be discussed. The section titled "A Poison from the Garden" will cover the toxin isolated from the Castor bean of the Spurge family. The last section, "Especially Potent Poison," will focus on an especially nasty poison of the de' Medici era: strychnine.

Key Words: Poison, Curare, Coniine, Turbocurarine, Circutoxin.

BACKGROUND

From prehistoric times to the present, people have used (and abused) toxic chemicals. A few [1] of the many toxicological landmarks might include: a) The Ebers Papyrus (c.1500 B.C.) which described many known poisons of that time, b) The Book of Job (c. 400 B.C.) which described poison arrows, c) Hippocrates (c. 400 B.C.) who described some therapeutic uses for toxins, d) Theophrastus (c. 300 B.C.) who made a record of poisonous plants, e) Sulla (c. 82 B.C.) who wrote the first law against poisoning, f) Dioscorides (c.60 A.D.) who made a classification of plant, animal, and mineral toxins, g) Maimonides (c. 1200 A.D.) who wrote a paper on the treatment of poisoned patients, h) Catherine de' Medici (c. 1580 A.D.) who kept a diary in which she recorded dosages, symptoms, time frames, and complaints of people she experimented on with different poisons, i) Paracelsus (c. 1567 A.D.) who published a treatise on the occupational cause of miners' disease, j) Percival Pott (c. 1775 A.D.) who published a paper on the carcinogenicity of soot (polyaromatic hydrocarbons) in chimney sweeps, k) Orfila (c. 1825 A.D.) who developed forensic chemical tests for poisons and used them in legal cases, l) Schmiedeberg (c. 1880 A.D.) who trained numerous toxicology students who then worked in pharmacology and toxicology laboratories, m) the introduction of laws governing the use and regulation of anesthetics, disinfectants, patent medicines, drugs, food additives, cosmetics, pesticides and fungicides (c. 1850-present), n) the developmental birth defects among the offspring of mothers who had used thalidomide (c. 1960s) which led to legislation banning the use of the drug and requiring pharmaceutical industries to do multi-species animal testing on drugs prior to marketing them, and o) the publication of *Silent Spring* by Rachel Carson (c.1962) which led to laws governing the use of environmental toxins and toxicants. It should also be noted that wars have

led to the development and use of toxic chemicals for military gain. A few 20th century examples [2] would include chlorine, mustard, tabun, sarin, soman, and lewisite gases, radioactive chemicals such as uranium and plutonium, and biological agents such as *Y. pestis* and *V. cholerae*. New agents (e.g., Agent Orange) and old ones (e.g., rye ergot), they have all made their mark on history.

During the 16th to the 18th century, history was riddled with the use of poison by a number of prominent individuals for a host of reasons spanning from infidelity to inheritance. Two of the most ruthless poisoners included the Marquise of Brinvilliers, whose exploits were so astounding that famous author Alexandre Dumas wrote a novel based on true events surrounding her life [3-4]. This "Lady of Poison" was proven to have poisoned the majority of her family for the family inheritance [4]. However, she first tested her poison on the patients of the surrounding hospitals to test the potency of the poison before administering the poison to her family [4]. She is even rumored to have poisoned her very own daughter for the crime of being a simpleton, though she did instantly regret the act and quickly administered the antidote … milk [4]. Upon the death of her lover, a military man and ex-convict, Sainte-Croix, a chest was found containing correspondence between himself and the Marquise that told of all her exploits [4]. The Marquise was then captured, tortured, tried, beheaded and burned in 1676 [4]. It was said that the crowd that had gathered to witness her burning danced in her ashes as they blew over the crowd [4].

Another woman of renowned talent in the art of poisoning was Catherine Deshayes [4-5]. She was nicknamed "La Voisine," and at the time of her death she had confessed to a multitude of poisonings of jealous or unfaithful husbands, wives and lovers. She even confessed to the murders of many unwanted children birthed by unwed women [5]. She boasted of having poisoned more people than all the famous or professional poisoners of her era [5]. However, poisoning was not Deshayes' only method of killing unwanted children [5]. She was known for her practice in the dark arts, the occult, and Satanism [5]. It was rumored that if an unwed woman were to conceive, Deshayes' services would be given to the woman free of charge and would encompass free room and board while the woman carried the child to full term [5]. Upon the child's birth, Deshayes would then sacrifice the child to her deity [5]. It was even whispered that her stepdaughter was her apprentice and took part in the ceremonies [5]. Interestingly enough, the same official responsible for the capture, torture and justice of the Marquise de Brinvilliers in 1676, Officer Desgrez, was also the driving force behind Deshayes' downfall as well [5]. Deshayes was arrested and held in prison until all the aristocracy that had any connection with her were under protection, at which time she was then tortured and burned in 1680 [5]. She did not go gracefully into that good night. She died cursing all the aristocracy and their hypocrisy as well as all the accusing priests [5].

The big players of this poisoning era and probably the most renowned, were all members of the same family, de' Medici. Though the member most remembered for the family's talents with poisoning is Catherine de' Medici, the entire family partook of the dark art and often made their living from their knowledge of the art [3,6]. Her father, the Grand Duke of Cosimo I de' Medici aided in the assassination of Piero Strozzi by sending an aid the poison, along with instructions, to poison Strozzi's wine bottle in 1548 [6]. Ferdinando de' Medici, Cosimo's son, is rumored to be connected to his own brother's, Francesco I de'Medici, and his wife, Bianca Cappello's, death in 1587 in order to gain the Granducal throne [6]. Though originally, the two were declared to have died from malaria, Francesco first followed by his wife a few days later, modern day technology has allowed for bone testing for arsenic and proved that the 'grand-ducal couple" did in fact die from arsenic poisoning and not malaria [6-7]. Though, what can be expected when after the death of the couple, Ferdinando was placed in charge of the autopsy and all examinations and investigations into the couples death [7]? Then there was dearest Catherine, who, in truth, depending on whose account you read, tells two completely different sides of the same coin.

This rampant use of poisoning for political reasons, personal vendettas and power struggles had everyone of that era on edge. Kings were being warned that their own children were planning on not only poisoning them, but also their wives and all remaining children [6]. Queen Elizabeth I herself became the focus of a failed poisoning attempt from one of her own ladies-in-waiting [6]. The royal cook threatened to poison the food of King Henry IV of France and Maria de' Medici herself [6]. The threat of poisoning was everywhere. There is even one story of a minister of Spain becoming so paranoid that one night his steward did not rinse out his wine glass well enough to rid the glass of residual vinegar and salt giving the wine an odd taste sending the minister into a crazed panic and yelling for antidotes [6]. Even Cosimo I de' Medici himself was threatened to be wary of his own face towels [6].

While another of the de' Medici family, Giangiacomo de' Medici di Marignano, was also the target of a successful assassination via poisoning [6]. Though the de' Medici's style of poison and antidotes mainly stemmed from herbology, it was not the only form of that time. There was a case in 1568, where a fourteen year old girl ground up mercury mirror glass into a fine powder and seasoned her family's salad with the powder all because her parents were going to send her to a convent [6]. Also, not all poisons and antidotes came from the de' Medici family. In 1660, there was a Roman priest that used the poisons and antidote from a woman known as Poisonous Girolama in order to make his "miraculous cures" seem more fantastical [6].

Though all poisons known and utilized during this era are not all plant derived (i.e., arsenic, which is a metal based poison), herbology was the main practice for deriving poisons and many could be found right outside, even homegrown. Included in this paper only a scant few of the deadly poisons derived from plants will be discussed. Though some may not have been used in the de' Medici era of poisoning, it is quite possible that some actually were. For example, "a description of 'horribly deformed corpses' and 'profuse bleeding from all orifices'" leads one to wonder, could strychnine poisoning have been the culprit [6]?

POISONS FOUND IN THE WILD

Curare Plants

The plants containing the curar toxin reside in the Loganiaceae and Menispermaceae families [8]. These 'curare plants' contain a toxin known as tubocurarine (Bowman, 2006). This toxin resides in all parts of the plant and is obtained by boiling the roots, bark and stalks of the plant [8]. Two species of plants are used to isolate tubocurarine: *Chondrodendron tomentosum* and *Strychnos toxifera* [9]. This toxin is a member of the quaternary alkaloids (meaning it is an alkaloid structure that contains four R groups branching from its core structure) and acts as a neuromuscular junction blocker [8]. Though one of the R groups of the alkaloid contains one nitrogen that is tertiary, this nitrogen is charged at physiological pH and therefore keeps the classification of the molecule as a quaternary alkaloid [9]. This toxin was first studied by Claude Bernard in the mid-19th century, but the South American tribes and the Amazonian tribes had been using this toxin for centuries to poison the tips of their arrows for hunting [9]. The amount of toxin used was defined by how many trees a monkey could climb or swing to after poison exposure [9]. If the monkey could climb a tree and swing to two more trees it was considered a weak dose of toxin, but if the monkey was paralyzed while climbing up the initial tree, then it was considered to have been injected with a strong or potent dose [9]. Tubocurarine is highly potent when injected into the blood stream and has very little absorptivity if ingested, which is why the tribes can still eat the meat killed in this manner and not poison themselves in the process [9]. However, this toxin is actually used in present day as a paralytic before certain types of surgeries for intubation, a treatment for tetanus, polio, spastic cerebral palsy and myasthenia gravis [9-10].

Tubocurarine is considered to be classified as a nondepolarising blocking toxin classified as a *leptocurare* [9]. In essence this poison blocks the transmission or communication of the acetylcholine receptors on the postjunctional face of the motor endplates of striated muscles. One study shows tubocurarine to not only block acetylcholine from interacting with the acetylcholine receptors, but to also block muscular responses to nerve stimuli [9]. This blocking mechanism is strong enough to continue to block this acetylcholine/receptor interaction such that even when a stimulant was introduced into the system, the blocking mechanism remained strong [9]. This potent blocking mechanism is believed to be contributed to the actual structure of the compound [9]. Tubocurarine's structure is shown in Figure 1.

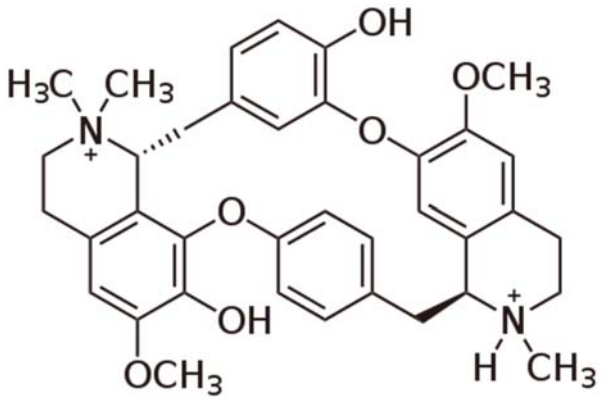

Figure 1. Turbocurarine
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This structure is what one study believed to be a perfect example of the relationship between quaternary ammonium function and neuromuscular blocking ability [9]. The compound contains two positively charged nitrogens, even though one is a tertiary amine, giving the structure two quaternary nitrogen centers which is a concept has been thought to lead to more potent compounds [9]. This stoichiometry allows for a more flexible structure which is what this particular study believes to be a major factor in tubocurarine's ability to bind with strong affinity and secure the receptor from binding with acetylcholine [9]. It out binds acetylcholine because that second positively charged nitrogen center will bind to another site close to the receptor (an accessory site) that plays no role in acetylcholine binding, giving it an increased chance of securing the receptor nearby [9].

This second type of receptors is termed nicotinic receptors [9]. These types of receptors are blocked by reversible neuromuscular blocking toxins (i.e., nondepolarising toxin) [9]. These receptors are found on the motor nerve endings [9]. These receptors are selective for Calcium when in their open state [9]. These nerve endings are referred to as nerve terminal autoreceptors [9]. These autoreceptors are believed to have a role in mobilizing acetylcholine from reserves in order to handle an increased frequency of nerve impulses associated with transmissions needed for striated muscle excitement [9]. Tubocurarine has been shown to block these autoreceptors, creating a 'tetanic fade' [9]. This tetanic fade is defined as the muscle's inability to maintain a muscle contraction no matter how high the electrical impulse generated [9]. This inability to maintain a contraction means that the muscle is at a constant relaxed state (i.e., paralysis) [9]. The effects of tubocurarine can be counteracted via acetylcholinesterase inhibitor administration or an induced dose, production, or release of increased levels of acetylcholine [9].

Under the workings of the above mechanisms, if de' Medici or one of the other royal ladies of the court had used tubocurarine on one of her unsuspecting guests, they would have first witnessed a paralysis of the victim's eyes, nose and neck. Paralysis of the victim's limbs would have followed. The last remaining and arguably the most important muscle to become paralyzed under the tubocurarine influence would be the diaphragm, leading to respiratory distress, which would have ceased the victim's breathing resulting in death via asphyxiation [8, 10].

Poison & Water Hemlock

Though it is assumed that "a hemlock plant ... is a hemlock plant ... is a hemlock plant", in the parsley family Apiaceae/Umbelliferae, this method of logic is not necessarily true [10-13]. Yes, both poison hemlock and water hemlock are highly poisonous, but the actual toxins contained in the two different plants differ. The two plants also differ in which part of the plant contains the toxin(s). Poison hemlock's toxins are throughout the entire plant, making the entire plant poisonous whereas water hemlock holds its toxin in the roots and stem (tubers) of the plant, making just those parts of the plant toxic with the tubers being twice as toxic as the roots [12-13].

Poison hemlock (*Conium maculatum*), which was used to kill Socrates in 399 B.C. in Athens, to poison political prisoners in ancient Greece, and believed to have possibly been given to Jesus Christ along with vinegar and myrrh at the time of His crucifixion, contains eight alkaloids, but there are two dominant piperidine alkaloid players: coniine (see Figure 2) and γ-coniceine [10-13]. These two toxins are formed from an eight-carbon chain from four acetate units that become cyclical [11].

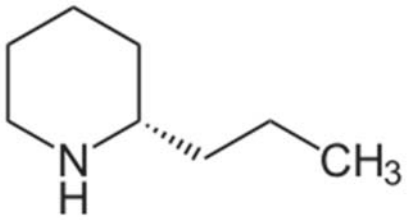

Figure 2. Structures of coniine Permission allowed through public domain, retrieved from http://en.wikipedia.org/wiki/Coniine#mediaviewer/File:Coniin_-_Coniine.svg

Coniine is more prominent throughout the plant during the seed and mature stages of the plant's life cycle and is the toxin most commonly found in *dried* poison hemlock [12-13]. Γ-coniceine is the dominant toxin present during the plant's vegetative stage of life and is tested to be eight to ten times more toxic than coniine [12-14]. It is believed that as poison hemlock dries out, there is an alkaloid "shift" from γ-coniceine to coniine [14]. These two toxins are so potent that an individual can receive a fatal dose by simply eating an animal that has ingested the two toxins [13]. The mechanisms of these two alkaloids are

also nondepolarising neuromuscular blockers like their sister tubocurarine [15]. Though the effects of these two toxins are two fold, as tubocurarine is, their main focus is on the nicotinic receptors on the motor nerve endings and by blocking spinal reflexes via how they affect the medulla [11,15]. These two toxins also affect the autonomic ganglia and can overstimulate cholinergic receptors, which is why these sister toxins are considered to be biphasic in that there exists two distinct phases of reactions of the central nervous system to the alkaloids [15]. The first effect causes a stimulation of the skeletal muscles during an early nicotinic effect which is considered the initial central nervous system stimulation [12]. This stimulation is then followed by a series of seizures of progressively intense constrictions with intermediate periods of relaxation with each period of relaxation being of shorter duration than the previous [12]. This series of seizures is then followed by a relaxation of these same muscles due to the blocking of the nicotinic receptors which is the later or delayed relaxation of the central nervous system [11-12]. Poison hemlock poisoning can also lead to muscle damage which explains the raised values of muscle enzymes (lactic dehydrogenase, aspartate aminotransferase and creatine kinase), elevated liver function values and myoglobinuria [12, 15]. One study theorized that the biphasic nature of the sister toxins could actually be dose dependent. For example, at low toxic doses, a blocking of spinal reflexes by the toxins and their effects on the spinal cord cause the initial stimulation and then the secondary phase, the relaxation action, is due to the toxins' effect on the autonomic ganglia [12]. Then at larger doses, the initial stimulation phase stimulates the skeletal muscle and the relaxation phase is cause by the subsequent neuromuscular blocking mechanism [12].

Each responsive phase of poison hemlock poisoning comes with its own set of symptoms. The symptoms of the initial phase of stimulation (effects of autonomic ganglia interaction) display within fifteen to sixty minutes after ingestion and consist of: nausea, vomiting, frothing, nervousness, bronchorrhoea, hypertension, tachycardia, agitation, ataxia, confusion, muscle fasciculation, burning of the mouth, throat and abdomen, excessive salivation, trembling, loss of coordination (stumbling), dilation of pupils, mydriasis, impaired consciousness and myalgias [11-15]. The symptoms of the secondary phase of relaxation (effects of overstimulation of cholinergic receptors) consist of: diarrhea, aponea, bradycardia, hypotension, muscular weakness, muscle paralysis, lethargy, weak or slow heartbeat, headache, ataxia, coma, rhabdomyolysis, acute tubular necrosis and respiratory muscle paralysis due to phrenic nerve paralysis, often defecation and urination, and "mousy" odor to the breath, urine and sweat and eventually death from respiratory paralysis [11-15].

Poison hemlock toxins are also considered to be teratogenic toxins. This means that low constant doses of poison hemlock toxins during the gestation period of fetal development will cause deformities and abnormalities in a fetus [11-15]. These birth defects include arthrogryposis, scoliosis, torticollis, excessive flexure of the carpal joints and cleft palate [12]. This teratogenic property is believed to be due to the length of the side chains and the amount of unsaturation of the carbon bindings in the piperdine rings of coniine and γ-coniceine. Teratogenicity is usually associated with a saturated ring and a propyl or longer side chain [12].

At present there is no counteracting therapy for poison hemlock poisoning. The only aid to those unfortunate enough to ingest the toxic plant is gastric suction, administration of activated charcoal if the patient is not yet comatose, and diuresis to prevent possible renal failure due to rhabdomyolysis and myoglobinuria [11, 15].

Water hemlock (*Cicuta verosa*), though from the same family as poison hemlock, contains cicutoxin, which is a toxin that acts directly on the central nervous system, but is not an alkaloid [11, 15]. This toxin is a long double and triple bonded (i.e., highly unsaturated) carbon chain alcohol and is shown below in Figure 3. This toxin is believed to be the most violently poisonous of all toxins [11-12].

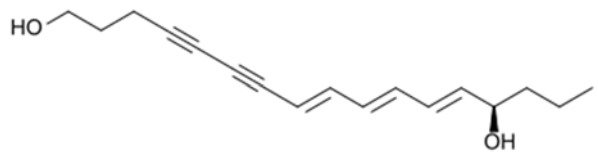

Figure 3. Circutoxin
Permission allowed through public domain, retrieved from http://commons.wikimedia.org/wiki/File:Cicutoxin.svg

Cicutoxin is not biphasic like the sister toxins of poison hemlock, though the convulsive episodes with alternating relaxation pattern does occur [14]. As stated above, cicutoxin acts exclusively and potently on the central nervous system [11, 15]. Symptoms of cicutoxin

poisoning consist of: seizures that often lead to lacerations on the tongue, cheeks, and lips as well as injuries to the head and limbs [12]. During these violent convulsions the head typically arches backwards and the legs stiffen [12]. The eyes, though closed tightly along with the mouth, display dilated pupils [12]. During the periods of relaxation there is an increase in body temperature (hyperthermia) and a chewing or grinding of the teeth [12]. This seizure/relaxation pattern usually ceases with a final paralytic seizure ending in anoxia and death [12]. As in the seizure/relaxation periods with the sister toxins of poison hemlock, each period of relaxation is shorter than the preceding period of relaxation [12]. The only treatment for these seizures is diazepam or phenobarbital and fluid replacement [11-12].

Flashing back to the era known for rampant poisoning, if one of the ladies of the court had decided to use one of the hemlocks, poison hemlock for this scenario, on a targeted enemy, the poisoner would have the added benefit of the poison not taking affect for fifteen to sixty minutes after their victim had ingested the poison depending on the dosage administered. The 'Lady of Poison' would have seen her victim first manifest signs of discomfort due the burning sensation of the mouth, throat and stomach at the initial ingestion of the poison. This would have been followed by the victim's increase in heart rate and breathing. Frothy saliva would begin to appear at the victim's mouth as would a slight tremor and loss of coordination of the limbs become detectable. The victim would then begin seizing violently before relaxing for a short period of time before seizing again. This pattern would only cease once the victim suffered a final seizure and all muscles in his body began to relax to such an extent that even the victim's breathing would cease. Once again, though more brutal than tubocurarine poisoning, the outcome would still be the same, a few more years in power for the 'Lady of Poison.'

A POISON FROM THE GARDEN

Castor Bean

Though the above mentioned poisons are extremely potent, it would seem the toxin of choice today is the toxic lectin ricin that is found in the beans of the castor bean plant, *Ricinus communis* and can be ground into a fine white powder that is easily soluble in water and can remain stable in a wide range of pH's [16]. This particular toxin has found its way onto the Centers for Disease Control and Prevention's (CDC) bioterrorist list [16-17]. It is classified as a Category B bio-agent and a weapon of mass destruction due to its availability, ease of isolation and production, its stability, lethality, its ability to be rapidly metabolized by the body and can be administered via ingestion, injection or inhalation [17-19]. This toxin only needs two to eight seeds, roughly 500 μg, to be ingested in order to administer a lethal dose [17,19]. The toxin is released when the hard seeds are cracked open during chewing and once released into the body, the resulting protein synthesis inhibition leads to cell death [17]. This toxin is present in all parts of the plant but the seeds contain the highest concentration (1% to 5%) [17,19]. This poison was made famous in 1978, during the Bulgarian Georgi Markov murder case or as some may know it … the "Umbrella Murder" [19]. Though this case may have been the one to make ricin famous, ricin's infamy did not stop there. There were two cases in 2002, three cases in 2003, and one case each year from 2004 to 2008 where this dangerous toxin was found in the wrong hands [19].

This toxic lectin is composed of a two-part stoichiometry that allows for one heck of a "wham-bamm-thank-ya-ma'am" tag team effort to disrupt the body's biological functions on a cellular level. Ricin is considered a heterodimeric type-2 ribosome-inactivating protein (RIP – aptly named) [19]. This type 2 configuration holds both an A chain, which serves as the actual ribosome inactivating enzyme, and a B chain that is a galactose/ N-acetylgalactosamine-binding lectin [18-19]. These two chains are covalently linked via a disulfide bond [19-19]. This B chain is inactive by itself but when linked to the A chain it serves as a method of entry into the cytosol of a cell [19]. This cytosol entry is dependent upon hydrogen bonding between the amino acid residues of the B chain and the complex carbohydrates on the surface of eukaryotic cells that have either N-acetyl galactosamine or β-1,4-linked galactose residues on their terminal end [19]. An illustration of the ricin protein is seen in Figure 4.

Once this protein enters the cytosol of a eukaryotic cell, it is engulfed by endosomes and hand delivered to the Golgi apparatus [19]. The lysosome and endosomes that would normally digest foreign proteins are harmless to ricin due to its ability to withstand an increased pH range [19]. Once at the Golgi, ricin uses retrograde transportation to travel through the Golgi and gain entrance into the endoplasmic reticulum (ER) [19]. This is where the A chain does its worst. Once inside, the A chain begins to inhibit all protein synthesis in the ER by inactivating all of the present ribosomes by binding a specific adenine ring

and depurinating it [19]. This action is irreversible [19]. This ring then gets pushed between two tyrosine rings and is hydrolyzed via N-glycosidase (26). This stops all elongation of any polypeptides which kills the cell [19]. As if this mechanism were not an instant death sentence, ricin infestation can also cause apoptosis, cell membrane damage, function and structure alterations and cause a cytokine storm that would bring about an inflammatory response from nearby cells [19]. Ricin is such a destructive protein that it only takes one single protein entering the cytosol in order to stop 1500 ribosomes per minute from functioning until the cell dies [19].

Symptoms of ricin poisoning depend on the dosage as well as the method of entry. If ricin were inhaled, symptoms would appear within the first eight hours whereas if the toxin were ingested then the first symptoms may display within the first six hours [19]. However, if inhaled, there is a possibility that respiratory symptoms may not manifest for the first 20 to 24 hours after inhalation [19]. Death usually occurs within thirty-six to seventy-two hours after exposure [19].

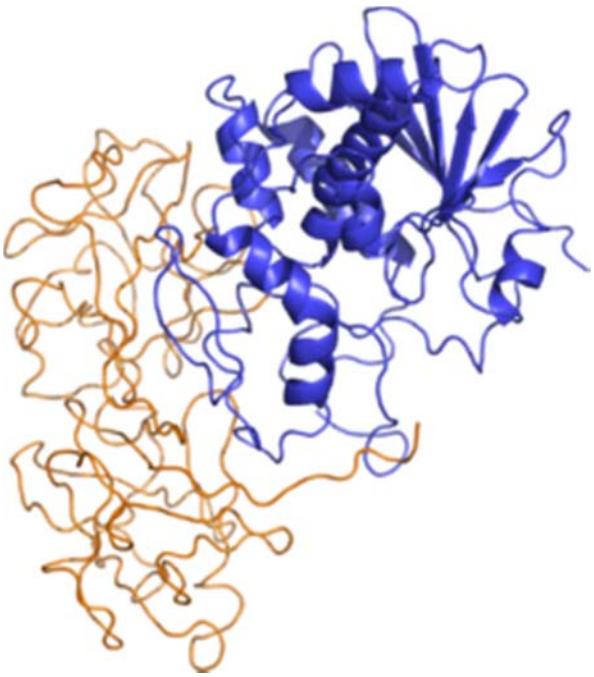

Figure 4. Ricin structure. The A chain is shown in blue and the B chain in orange. Permission allowed through public domain, retrieved from http://en.wikipedia.org/wiki/Ricin

If ricin were ingested, symptoms would consist of vomiting and diarrhea [19]. Both may become bloody and become accompanied by bloody urine as the toxic condition begins to cause gastric bleeding [16, 19]. This would lead to massive dehydration, low blood pressure, hallucinations and seizures [19]. This condition would lead to massive organ failure and result in death [19].

If ricin were inhaled, symptoms would begin with respiratory distress, fever, nonproductive cough, nausea, tightness of chest, heavy sweating, possible fluid retention in the lungs causing the skin to turn blue due to lack of oxygen, low blood pressure and finally respiratory failure or cardiac arrest leading to death [16, 19]. With this route of entry, the potency of the toxin depends on the protein synthesis inhibition, cytokine storm severity, and epithelial membrane injuries [19].

If ricin were injected, as in the "Umbrella Murder", symptoms could be delayed for ten to twelve hours and present with a fever, headache, hypotension, anorexia, abdominal pain and finally followed by multi-organ shut down [19].

Like the previous toxins, there is no medical treatment only symptomatic and supportive treatment. Gastrointestinal suction and activated charcoal administration along with fluid replacement, vasopressor, and electrolyte replenishing therapy are considered the main treatments for ricin poisoning [19]. For treatment of inhaled ricin, oxygen, endotracheal intubation and bronchodilators are suggested [19].

Assuming that the ladies in the 16th to the 18th century were not capable of aerosolizing or injecting ricin, it is assumed that if ricin was chosen to poison an enemy it would be delivered via the oral route. This type of route would be slow acting and the poisoner would have to go on faith that the ricin would have an effect on the victim. Within six hours the victim would start to complain of nausea, diarrhea and vomiting. These symptoms would continue to an extent that all excretions would begin to become bloody. This massive fluid loss would lead to dehydration which would lead to possible hallucinations and low blood pressure. As the victim's condition worsened, his organs would begin to shut down until shock set in along with seizures culminating in death.

ESPECIALLY POTENT POISON

Strychnine

Strychnine is another alkaloid (see Figure 5) that is similar to the curare plants in its mechanics, but this

particular plant is fatal in exceedingly small doses. The toxin is isolated from the seeds of the *Strychnos nux-vomica* tree of Asian-Indian origin, *Strychnos toxifera*, a plant of South American origin, *Strychnos spinosa*, a plant from the tropics of Africa or *Strychnos potatorum*, a plant of Indian origin [10, 20-22]. For the remainder of this section the focus will be on the *Strychnos nux-vomica* tree whose seeds contain 1.1% to 1.4% strychnine [21].

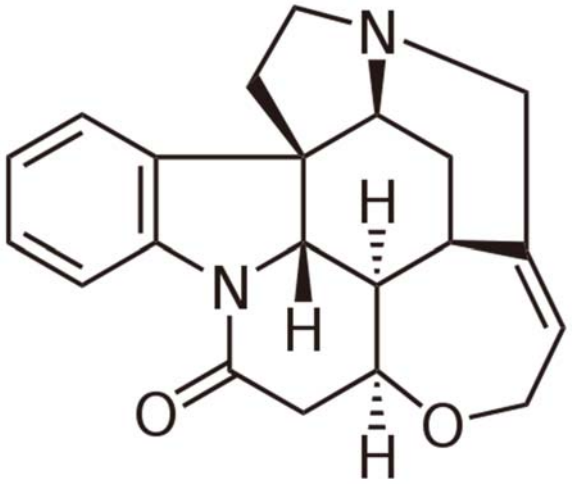

Figure 5. Strychnine
Permission allowed through public domain, retrieved from http://commons.wikimedia.org/wiki/File:Strychnine2.svg

Strychnine is a stimulant to the central nervous system (CNS) that causes muscle spasms, convulsions (seizures) that are triggered by the toxins ability to magnify the individual's sense of touch, olfactory, hearing and sight [10, 21-22]. These heightened sensations are what cause these seizures to often be re-occurring with only a short duration of exhausted relaxation between episodes [10, 21].

In essence, strychnine is a competitive/non-competitive antagonist to glycine for the inhibitory neurotransmitter receptors positioned in the spinal cord and the brain stem [21-22]. This blocking of a natural neurotransmitter inhibitory (glycine) results in an increase in muscular activity due to the unchecked increase in the activity of the neurontransmitters in the spinal cord and brain stem [22]. This increase in muscular activity can lead to: an increase in lactic acid resulting in metabolic acidosis, hyperthermia from the heat generated via constant muscle contraction from the increased motor neuron stimulation or rhabdomyolysis that leads to myoglobinuria that can result in renal failure [22]. The two most detrimental muscles to be affected by this toxin are the respiratory and cardiac muscles. This toxin is often lethal due to its ability to cause spasms of the respiratory muscles leading to respiratory arrest that would then lead to death via asphyxiation. If this toxin acted on the cardiac muscle, an individual would suffer from irregular or rapid heart palpitations followed by cardiac arrest culminating in death [22].

There are three possible mechanisms for this alkaloid. Two of the mechanisms are post-synaptic, but differ in that one mechanism is believed to be competitive while the other is believed to be non-competitive [23-24]. The remaining mechanism, while still seen as a theoretical possibility, displays a non-competitive pre-synaptic binding that seems to contain a trickle-down effect [23].

The first mechanism to be discussed will be the widely studied post-synaptic competitive mechanism. In a normally functioning system, glycine binds to an inhibitory receptor located on motor neurons called Renshaw cell-motor neurons located on the neuron's synapses [20-21]. When this glycine-inhibitory binding occurs, the motor neuron firing necessary for muscle contraction is inhibited allowing for relaxation [21]. In the competitive post-synaptic mechanism of strychnine, strychnine competitively competes against glycine for these receptor sites located on the inhibitory receptor sites on these motor neurons [21]. This action not only stops glycine from binding the inhibitory site, but also decreases the amount of glycine released from these sites which creates a loop ultimately lacking in any inhibitory action [21]. An interesting fact discovered in one study showed that upon the initial appearance of strychnine in the system, there was no change in glycine binding. It was only until there was a specific level of strychnine present that strychnine binding overpowered glycine binding [23]. Once at this specific level of surrounding strychnine, the strychnine out-bound glycine 2 to 1 and only continued to increase in strychnine's favor as the level of strychnine in the system increased [23]. This may mean that these targeted motor neurotransmitter receptors are actually more sensitive towards strychnine than glycine [23].

This induced lack of the inhibition of motor neuron firing leads to excessive motor neuron activity, inducing convulsions and increased muscular contractions [21]. Another side effect of this neuron overstimulation is that the individual's sense of hearing, sight, touch and smell are also over-sensitized [10, 21]. This heightened

sensitization of the senses means that even a slight stimulus to any of these senses has the ability to trigger reoccurring seizures and convulsions [10, 21].

The next mechanism is very similar to the previous one and leads to the same end result; only in this scenario, strychnine performs as a non-competitive inhibitor of glycine inhibitory binding to the motor neurotransmitter synapsis [24-25]. This non-competitive action is due to the belief that strychnine binds to a site that shares a slight overlap with the glycine receptor site, but that in fact two distinct binding sites exist [24-25]. This theory bases itself on the understanding that glycine inhibition acts via a pentameric chloride channel protein that consists of both α and β subunits [24]. These two subunits display a homology to the subunits of both γ-aminobutyric $acid_A$ ($GABA_A$) and nicotinic acetylcholine receptors [24]. These three receptors also share a quaternary structure, which is a common aspect among this particular group of neurotransmitter gated ion channels [24]. This same study proved that the α-subunit of the glycine receptor can change formation and become more sensitive towards strychnine instead of glycine leaving these ion channels open for a virtual chloride ion free flow leading to an increase in muscle excitation and contractions [24]. This study proved that under this model, strychnine was three magnitudes higher in binding potency compared to glycine [24]. However, the same study showed that if there was an increase in glycine levels, then increased binding of glycine would occur [24]. This lead to the belief that each receptor may contain more than one binding site leaving glycine and strychnine to compete for the same receptor just utilizing two distinctly and conformationally different, yet overlapping binding sites [24]. Though it is generally accepted that agonists of a particular receptor site induce a larger conformational change in the site over that of the change induced by the antagonist to that site, it would seem that this may not be true in this case since glycine is more readily disassociated from its receptor site as opposed to strychnine, which once bound has a tendency to stay bound to its receptor site [23-24]. This ability of strychnine to stay bound may be due to residual binding of charged amino acids (histidine around the binding sites that underwent conformational changes upon strychnine binding and the tyrosine or cysteine residues associated with the N-terminal region) associated with the receptor site to the charges of the strychnine molecule [24]. All of these amino acids, histidine, tyrosine, tryptophan and cysteine contain α-subunits homologous to the α-subunit of the nicotinic acetylcholine receptor [24]. Arginine residues with a positively charged side chain can also increase strychnine binding via the molecular interaction with strychnine's carbonyl group [24].

The final proposed mechanism of strychnine is a pre-synaptic effect on the actual release of inhibitory transmitters which in turn reduces the inhibitions of the collateral and VIII nerve [23]. This would mean that strychnine would inhibit the release of acetylcholine from the cervical ganglion into the peripheral nervous system, leading to strychnine having an indirect effect on the cholinergic nerve endings which may explain the hypersensitivity aspect of strychnine poisoning due to the increased nerve activity from the lack of inhibitory transmitters released from the ganglion [23].

Strychnine is a colorless, bitter tasting and odorless powder that is readily absorbed via the gastrointestinal tract, respiratory tract and via the skin [21-22]. The symptoms of strychnine poisoning via ingestion usual manifest within ten to twenty minutes post ingestion with cardiac or respiratory arrest occurring thirty five minutes after ingestion [22]. When strychnine is absorbed via the mucous membranes (i.e., intranasal or subcutaneously) symptoms can manifest as soon as five minutes to one hour after exposure to the toxin depending on the dosage. Due to strychnine's ability to rapidly metabolize (15% of all metabolized strychnine being in the form of strychnine N-oxide) in the body and its seemingly accumulative effect on the body's inhibitory processes, the toxin is shown to only have an internal half-life of ten to sixteen hours with 20% being excreted via the urine in an unchanged state [21-22].

Treatment for strychnine poisoning is tricky and depends on the route of exposure and the dosage received [21]. Once symptoms begin the individual should be moved to a dark, quiet room if a sensory deprivation chamber is not available [21]. An individual with strychnine poisoning should not be intubated and nasogastric lavage should be avoided due to the sensation possibly triggering a seizure [21]. Induced vomiting should also be avoided due to danger of asphyxiation should an onset of contractions activate during the actual vomiting [21]. Benzodiazepines (i.e., anti-spasmodics) or short acting barbiturates should be administered intravenously to stop or lessen the muscle contractions [20-21]. If the above medications are not sufficient, then heavy sedation or induced paralysis via pancuronium or vecuronium (known neuromuscular blockers) should be induced and the individual intubated at this point [20-21]. Common complications with strychnine poisoning are: respiratory or metabolic acidosis, muscle damage causing myoglobinuria leading to rhabdomyolysis culminating in

renal failure [21]. If hyperthermia goes untreated, the individual could sustain anoxic brain damage along with multi-organ failure and disseminated intravascular coagulation [21].

With the above in mind, if one of the devious ladies of the court were to choose this particular toxin to rid the court of an unwanted obstacle, the following would have been the final performance the victim would have performed on the esteemed "Lady of Toxin's" stage: In this scenario, it will be assumed that the route of exposure will be via ingestion since this route is easier to control than inhalation when in a crowded gathering. Within fifteen to thirty minutes the victim would begin to notice his senses of sight, hearing, olfactory and touch becoming more distinct than usual [21]. This increase in sensitivity would soon trigger the first of several clonic convulsions [21]. The victim's muscles would begin to rotate through rapid periods of contractions followed by relaxations giving the victim a "jerking" motion [21]. This convulsion would last for anywhere from thirty seconds to two minutes [21]. Once this initial convulsion ended the victim would only be allowed a moment's peace before the heightened sensations would trigger yet another clonic seizure. These seizures would start to become more tetanic in nature [21]. This means the victim would begin to display outward signs of sustained muscle contractions. These tetanic seizures would cause the victims heels and head to arch backward making the body form a large "bow" formation due to extreme hyperextension, a condition known as opisthotonus (4, 21]. During this time the jaws would become locked shut while the muscles of the face would also contract giving the victim the appearance of a grotesque grin, a condition known as "risus sardonicus" [21]. The eyes of the victim would assume a fixed stare with dilated pupils and can even move around in different directions as they begin to protrude from their sockets [21]. These tetanic seizures affect muscles of the diaphragm along with chest and abdominal muscles leading to respiratory distress causing anoxia and cyanosis [21]. Throughout this entire scenario the victim is fully conscious and in excruciating pain [21]. Once again, following each of these tetanic seizures is a period of complete muscle relaxation, allowing the victim to breathe normally relieving the cyanosis and pupil dilation and allowing the victim to fall into a deep exhausted sleep [21]. These relaxation periods would last for ten to fifteen minutes after which even the slightest stimuli to any of the heightened senses would trigger another tetanic seizure with each convulsive episode being more intense than the previous with a shorter duration of relaxation between each episode [21]. The victim will suffer approximately five of these episodes before expiring of respiratory arrest since very rarely does an individual live past five of these tetanic episodes [21].

CONCLUSION

In conclusion, the de' Medici era of poisoning was one filled with deceit, dangers, vendettas and fear. No one was considered safe from threats of dying violently. This paper only covered the mechanisms and symptoms of a scant few of the poisons that were running rampant during that time in history. In addition to poisons isolated from plants, there were a considerable number of poisons made from chemicals, heavy metals and even household items (i.e., the ground up mercury glass mirror) that were utilized during that era. Though the origins of the poisons changed, along with the method of delivery, the outcome was almost always the same … death. So next time you go to drink or eat after having left your seat at a dinner party, you may want to think about smelling your food or using that moment when you raise your glass during a toast to check for any "special additives" that may be awaiting your ingestion.

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THE MAKE UP OF YOUR MAKEUP

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Introduction

The manuscript is adapted from *The Make Up of Your Makeup* seminar that was originally presented at the #girlSTEM Conference on May 22, 2014 at Delaware Valley College in Doylestown, Pennsylvania. #girlSTEM seeks to inspire middle-to-high school girls in Bucks County, Pennsylvania to pursue Science, Technology, Engineering and Math (STEM) related fields. #girlSTEM features a variety of interactive seminars with professional women, hands-on workshops, and open forum discussions. This session explored making products with the young women based on a concept that was presented to their team using their knowledge from a list of ingredients and information about each of those ingredients. The attendees of #girlSTEM had an opportunity to explore the cosmetic industry from a drug development perspective in a way that engages them to dig deep into their purses and minds to examine what goes into the products that women use on the largest organ – their skin.

Abstract

The Quality by Design approach implemented in the drug development process is described by comparison to the intricacies of skin and cosmetic products as a framework for the manuscript. The interrelated aspects of material selection, process controls and product specification are discussed as they relate to the targeted product profile. The seminar encouraged the students to look closely at the materials that constitute the skin and cosmetic products they frequently use and the intended purpose of the ingredient to achieve the desired product functionality and attributes.

Key Words

STEM, Makeup, Quality by Design, Drug Development

The *Make Up of Your Makeup* is a high level perspective of how products for the skin are created and what role the ingredients play in meeting the intended product concept.

Discussion

Let us first take a look at how beautiful the skin is! Look around. The skin is not just the covering of our bodies, it is a working organ. The layers of your skin have individual functions as well as collaborative functions that help with defense, healing and internal controls. The skin has the innate response by the cells present to recognize and destroy foreign microbes. It is wonderful knowing that when you cut yourself, you can put a bandage over the cut and let you skin take care of the rest.

Since we are all scientists here, I am assuming that you are thinking the same questions that I am about the skin. If the skin performs all of these miraculous functions, why do we need beauty and drug products?

My response to that question is: since the skin is such a complex organ, there are so many different things that can possibly go wrong. That is why it is a challenge and there are countless opportunities to provide treatments.

For example, wound healing can be affected by several factors such as age, health and immune status. Typically, when we think of illnesses we consider those where a function is not working properly and therefore cannot fight disease or infection which can be described as hyporeactive immune response. There are a plethora of implications that are a response to excessive healing. Wound healing, particularly excessive wound healing as seen in eczema patients, can also be impacted by the interplay between genetics and environmental factors [1] to create a hyperreactive immune response for the patient. The drugs that are currently available for eczema function by slowing down the immune response of the patients to achieve the skin's normal ability to heal itself.

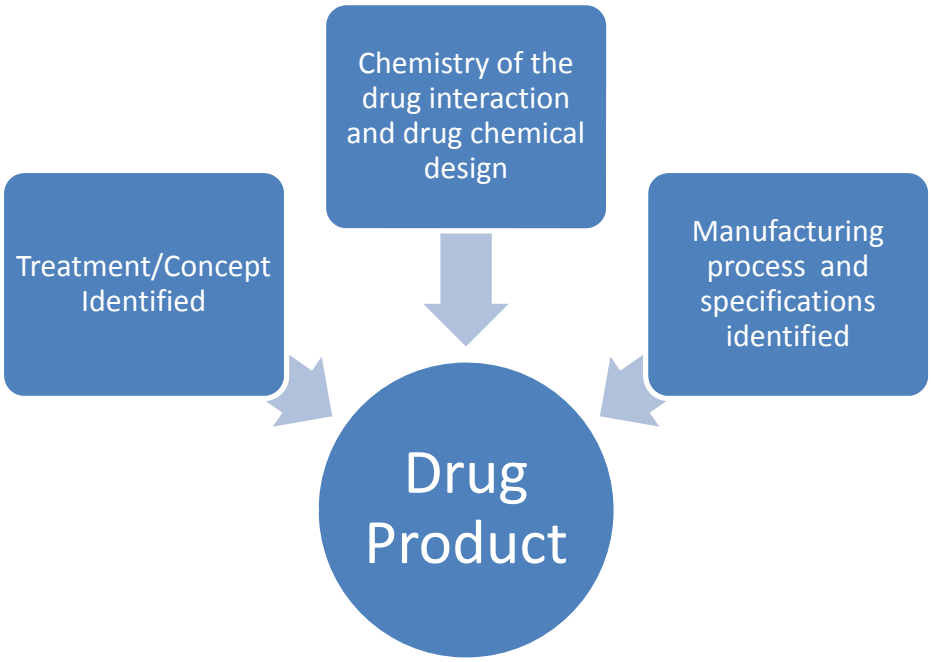

Figure 1. Overview of Drug Product Development Formulation Target Profile

The skin conveys inner health. There is a real connection between what's going on inside and outside the body. It is not really magic but rather that skin demonstrates some conditions that are going on inside the body to the outside of it. A skilled dermatologist can recognize the patient's disease status based on the manifestation of skin conditions [2].

In the context of this presentation, the general understanding described about the drug development focuses on the process which begins with an idea. A concept that is typically brought to the team and from that idea,

based on their knowledge of the mechanisms of the body, pharmaceutical chemistry, manufacturing and regulations, the team collaborates to create a project plan. The plan will move forward in the form of a compound (or multiple compounds) and manufacturing scheme that is identified to be tested clinically (Fig. 1). The goal of the team working together throughout the duration of the project is to design quality into the product through all aspects of drug product design. Once the formulation and process are found to be acceptable, the team will seek approval from the corporate leaders and the FDA to market the product.

The aim of pharmaceutical development is to design a quality product and its manufacturing process in order to consistently deliver the intended performance of the product. The information and knowledge gained from pharmaceutical development studies and manufacturing experience provide scientific understanding to support the establishment of the design space, specifications, and manufacturing controls [3].

There are a few ways the drug development team can approach the proposed drug concept. Again, the team will ask questions. What is currently in the market that can effectively treat the patient? Is there a drug product that exists that may currently treat the indication or a similar indication? Understanding the disease and specifically targeting indication as well as the pharmacology of the drug product and excipients is critical to the success of the development program.

Since many diseases currently have treatments which are clinically shown to be effective, drug development teams also look at the challenge in a different way. Can the drug currently in the market be improved? In the case of a painful skin condition, patients typically prefer a dosage that can be applied without causing the affected areas to itch or burn. Optimizing the application, excipients and dose of the product for this type of patient could potentially increase their quality of life.

When considering the formulation of the product for the proposed concept, the pharmacokinetics and pharmacodynamics must also be considered. If a product is being designed to treat a skin indication, the state of the skin is critical. If the skin is broken at the time of application, the route of dosing the product and other physical attributes become critical to the quality of the product.

For the product to work properly and for the patient to benefit from the product, the target product profile will be established by the development team. The team will choose raw materials based on the purpose of the product and known excipients for the type of formulation using the Inactive Ingredient search tool provided by the Federal Drug Administration [4].

Once the formulation is finalized and has been shown to meet all of the critical specifications for the product, the manufacturing process is validated and scaled appropriately. To seek approval from regulatory authorities to market the product to patients, the established process to execute the concept flawlessly and ensure that it is reproducible and maintains the specifications over time must be determined.

Summary and Recommendations

The students that participated in the #girlSTEM conference seminar were offered a unique perspective of

drug development using makeup as a mechanism to establish a direct connection to products the young women use every day. The young women were encouraged to look at the labels of their own skin products and evaluate the ingredients while using naturally derived ingredients to meet a target product profile in a hands-on activity. The young women were strongly encouraged to look differently at their skin and the products they use by applying their education and experience to reevaluate their definition of beauty and become strong women through mind, body, and spirit.

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Book Reviews

Organic Structure Analysis (Second Edition)

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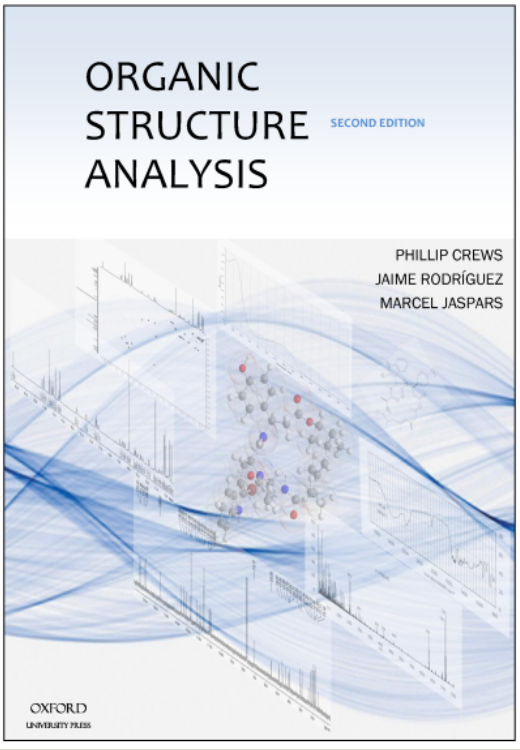
Many undergraduate chemistry programs are offering upper-level coursework in organic structure determination. This type of class is useful to upper-division students. The additional skills gained are applicable for other advanced courses, independent research projects, and future graduate studies.

The Crews text is very useful for a 400-level special topics class, particularly if the pedagogical style of the professor leans towards inquiry-driven or "flipped classroom" models of teaching. Many aspects of the text make it highly suitable for an introductory graduate or upper-level undergraduate course.

The book has a number of very useful tables, but this is not a reference book. Rather, it is instructional in nature. There are questions at the end of each chapter and a solutions manual provided to instructors. The solutions manual is valuable. Because the solutions are not to be found in textbook itself, the end-of-chapter questions are suitable for use as graded assignments. The chapters are laid out logically, beginning with a general introduction into the use of spectral data and a chapter on the basics of nuclear magnetic resonance, with subsequent chapters covering the most practical details of single and multidimensional NMR techniques. Other spectroscopic techniques covered in the book include mass spectrometry and infrared and ultraviolet spectroscopy. There are three chapters dedicated to mass spectrometry, with the general layout being similar to that for NMR (basic concepts, followed by detailed modern techniques and relevant applications of those techniques). The last two chapters are dedicated to the use of combined techniques in order to determine complex organic structures and are the capstone experience of a course of this type.



Authors:	Phillip Crews, Jaime Rodríguez, and Marcel Jaspars
Publisher:	Oxford University Press: New York
ISBN#:	978-0-19-533604-7
Year:	2010
Price:	$145

The text itself is readable, although still challenging for students whose last experience with spectroscopic structure interpretation was sophomore organic chemistry. What I particularly like about this book is that unlike sophomore-level, and even some upper-level, textbooks on this topic, the answers to questions are not simply to be found buried in the text. Enough information is generally given, but the students must engage themselves fully

in the "More Challenging Problems" in order to arrive at the solutions. There are also references for outside reading in many of the end-of-chapter problems. This feature can be a double-edged sword to smaller institutions that may lack access to a 1978 article from *Tetrahedron Letters*, for example. With advanced planning, however, an instructor could either take advantage of inter-library lending or skip a particular question.

When looking for resources for an organic structure determination course, one tends to find texts that are either somewhat oversimplified or overly detailed in the treatment of NMR principles. The Crews text offers a middle ground, where basic principles are covered at a high, but reasonable level and concepts are reinforced by challenging problems. This blend of explanatory text, extremely useful data tables and conceptual questions makes the Crews text a highly suitable choice for the upper-level undergraduate or beginning graduate student.

Metal-Polymer Nanocomposites

Reviewed by Dr. Kenneth Abate
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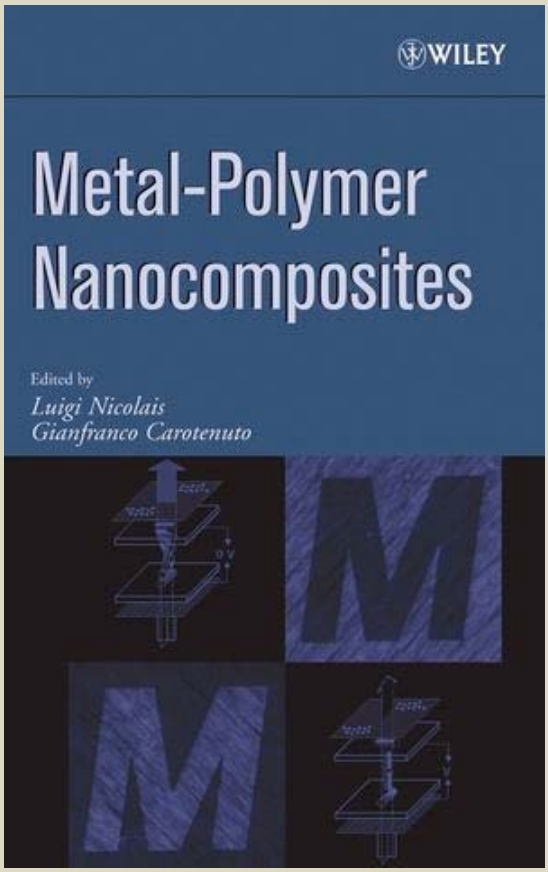
Metal-Polymer Nanocomposites contains nine chapters, each written by a different author or set of authors, most of whom are not American. Because of this authorship, the style differs with each chapter. Each chapter is a review of the technology on a specific area of nano-sized metal particles, materials containing nano-metal particles, and polymers containing nano-metallic particles and their properties.

The first chapter deals with general physical and chemical properties of nano-metallic particles and how and why the properties of nano-sized metal particles can dramatically differ from those of the same metal in bulk. It is followed by several chapters detailing the different synthesis methods commonly used to produce nano-sized metal containing polymeric systems and the properties of the materials produced. These methods include vapor-deposition cryochemical synthesis, the synthesis of metal containing polymers by pyrolysis of metal ion containing polymers and precursors, polymerization and copolymerization of metal containing monomers, and multilayer systems consisting of plasma polymerized thin films embedded with metal particles by simultaneous plasma polymerization. (The latter method produces a composite in which metal particles are embedded in one plane between two plasma polymer layers that do not contain metal particles.) The last few chapters are devoted to optical properties of metal-polymer nanocomposites.

In some of the chapters, there are brief discussions concerning practical applications of nano-sized metal containing materials. Among these are dichroic films, LCDs,



Editors:	Luigi Nicolais and Gianfranco Carotenuto
Publisher:	Wiley-Interscience
ISBN:	0471471313
Year:	2004
Price:	$99.95

color filters, polarizers, dyes, magnetic-resonance-imaging contrast agents, optical sensors, and non-linear optical devices. In general, the chapters are clearly, and thoroughly presented. The subjects covered are not for the casual reader and one should have a sound chemical background to fully appreciate and use the information presented.

The book is about 300 pages in length and of good quality. It is filled with diagrams, graphs, figures, pictures, and various formulations that are clearly presented and readily understood. The chapters have very extensive reference sections that overall cite literature from 1857 to 2003. Most references are from the 1990's. It is interesting to note that there are a few "spell check" type word errors in the chapters and, also, that four authors were required to write a thirty-page chapter. Although I would consider it to be expensive, *Metal-Polymer Nanocomposites* should be useful to those entering the field of nano-sized metal containing polymers and composites.

A Friend of Chemistry

Dr. P. V. Thomas

Dr. P. V. Thomas is the Founder and Director of the Thin Film Lab at Mar Ivanios College, Trivandrum, Kerala, India. He is a former professor and head of the Department of Physics at Mar Ivanios College and member of the Board of Studies (Nano Science and Nanotechnology), University of Kerala. He is a distinguished member on the editorial Review Board of *The Chemist* and a strong supporter of the journal. In addition to teaching thousands of undergraduate and graduate students over his career, Professor Thomas has carried out pioneering work in thin films and nanoscale material fabrication and photoluminescence studies with considerable overlap with chemical sciences.

His research interests include thin film fabrication by RF magnetron sputtering and sol gel methods, photocatalytic thin films of doped/alloyed TiO2 and ZnO2, rare earth doped luminescence thin films, and transparent conducting oxide thin films. Dr. Thomas has published extensively in highly regarded journals such as the *Thin Solid Films*, the *Journal of Nanoscience and Nanotechnology*, the *Applied Surface Science*, *The Chemist*, and the *Journal of Sol-Gel Science and Technology*. He has designed a dip coating system for thin film fabrication and RF magnetron for sputtering systems.

A physicist by training, Dr. Thomas received his bachelor's degree from University of Kerala, master's degree from Sardar Patel University and doctorate from University of Kerala, India. He is a member of the Materials Research Society and the Indian Vacuum Society. Dr. Thomas is a sought after guide for master's and doctoral research students, post doctoral students and university faculty on sabbatical. His Thin Film Lab has received guest scientists and visiting professors from Florida Atlantic University, USA and Advanced Research Chemicals, USA.

His pleasant personality is a magnet that attracts people of diverse backgrounds to engage in thought provoking discussions with him. Dr. Thomas is someone who is not prejudiced about one field or another, but rather someone who is genuinely interested in scientific inquiry towards the welfare of mankind. In fact, this openness makes Dr. Thomas unique among physicists who embrace chemical sciences in pursuit of their quest for understanding the world around. Indeed, Dr. P. V. Thomas is a highly dedicated distinguished scientist, and a friend of chemistry.

The AIC Code of Ethics

Approved by the AIC Board of Directors, April 29, 1983

The profession of chemistry is increasingly important to the progress and the welfare of the community. The Chemist is frequently responsible for decisions affecting the lives and fortunes of others. To protect the public and maintain the honor of the profession, the American Institute of Chemists has established the following rules of conduct. It is the Duty of the Chemist:

1. To uphold the law; not to engage in illegal work nor cooperate with anyone so engaged;

2. To avoid associating or being identified with any enterprise of questionable character;

3. To be diligent in exposing and opposing such errors and frauds as the Chemist's special knowledge brings to light;

4. To sustain the institute and burdens of the community as a responsible citizen;

5. To work and act in a strict spirit of fairness to employers, clients, contractors, employees, and in a spirit of personal helpfulness and fraternity toward other members of the chemical profession;

6. To use only honorable means of competition for professional employment; to advertise only in a dignified and factual manner; to refrain from unfairly injuring, directly or indirectly, the professional reputation, prospects, or business of a fellow Chemist, or attempting to supplant a fellow chemist already selected for employment; to perform services for a client only at rates that fairly reflect costs of equipment, supplies, and overhead expenses as well as fair personal compensation;

7. To accept employment from more than one employer or client only when there is no conflict of interest; to accept commission or compensation in any form from more than one interested party only with the full knowledge and consent of all parties concerned;

8. To perform all professional work in a manner that merits full confidence and trust; to be conservative in estimates, reports, and testimony, especially if these are related to the promotion of a business enterprise or the protection of the public interest, and to state explicitly any known bias embodied therein; to advise client or employer of the probability of success before undertaking a project;

9. To review the professional work of other chemists, when requested, fairly and in confidence, whether they are:
    a. subordinates or employees
    b. authors of proposals for grants or contracts
    c. authors of technical papers, patents, or other publications
    d. involved in litigation;

10. To advance the profession by exchanging general information and experience with fellow Chemists and by contributing to the work of technical societies and to the technical press when such contribution does

not conflict with the interests of a client or employer; to announce inventions and scientific advances first in this way rather than through the public press; to ensure that credit for technical work is given to its actual authors;

11. To work for any client or employer under a clear agreement, preferable in writing, as to the ownership of data, plans, improvements, inventions, designs, or other intellectual property developed or discovered while so employed, understanding that in the absence of a written agreement:
    a. results based on information from the client or employer, not obtainable elsewhere, are the property of the client or employer
    b. results based on knowledge or information belonging to the Chemist, or publicly available, are the property of the Chemist, the client or employer being entitled to their use only in the case or project for which the Chemist was retained
    c. all work and results outside of the field for which the Chemist was retained or employed, and not using time or facilities belonging to a client or employer, are the property of the Chemist;

12. Special data or information provided by a client or employer, or created by the Chemist and belonging to the client or employer, must be treated as confidential, used only in general as a part of the Chemist's professional experience, and published only after release by the client or employer;

13. To report any infractions of these principles of professional conduct to the authorities responsible for enforcement of applicable laws or regulations, or to the Ethics Committee of The American Institute of Chemists, as appropriate.

The Chemist
Journal of the American Institute of Chemists

Manuscript Style Guide

The Chemist is the official online refereed journal of The American Institute of Chemists (AIC). We accept submissions from all fields of chemistry defined broadly (e.g., scientific, educational, socio-political). *The Chemist* will not consider any paper or part of a paper that has been published or is under consideration for publication anywhere else. The editorial office of *The Chemist* is located at: The American Institute of Chemists, Inc. 315 Chestnut Street Philadelphia, PA 19106-2702, Email: aicoffice@theaic.org.

Categories of Submissions

RESEARCH PAPERS

Research Papers (up to ~5000 words) that are original will only be accepted. Research Papers are peer-reviewed and include an abstract, an introduction, up to 5 figures or tables, sections with brief subheadings and a maximum of approximately 30 references.

REPORTS

Reports (up to ~3000 words) present new research results of broad interest to the chemistry community. Reports are peer- reviewed and include an abstract, an introductory paragraph, up to 3 figures or tables, and a maximum of approximately 15 references.

BRIEF REPORTS

Brief Reports (up to ~1500 words) are short papers that are peer-reviewed and present novel techniques or results of interest to the chemistry community.

REVIEW ARTICLES

Review Articles (up to ~6000 words) describe new or existing areas of interest to the chemistry community. Review Articles are peer-reviewed and include an abstract, an introduction that outlines the main point, brief subheadings for each section and up to 80 references.

LETTERS

Letters (up to ~500 words) discuss material published in The Chemist in the last 8 months or issues of general interest to the chemistry community.

BOOK REVIEWS

Book Reviews (up to ~ 500 words) will be accepted.

Manuscript Preparation

RESEARCH PAPERS, REPORTS, BRIEF REPORTS & REVIEW ARTICLES

- **The first page** should contain the title, authors and their respective institutions/affiliations and the corresponding author. The general area of chemistry the article represents should also be indicated, i.e. General Chemistry, Organic Chemistry, Physical Chemistry, Chemical Education, etc.

- **Titles** should be 55 characters or less for Research Papers, Reports, and Brief Reports. Review articles should have a title of up to 80 characters.

- **Abstracts** explain to the reader why the research was conducted and why it is important to the field. The abstract should be 100-150 words and convey the main point of the paper along with an outline of the results and conclusions.

- **Text** should start with a brief introduction highlighting the paper's significance and should be understood to readers of all chemistry disciplines. All symbols, abbreviations, and acronyms should be defined the first time they are used. All tables and figures should be cited in numerical order.

- **Units** must be used appropriately. Internationally accepted units of measurement should be used in conjunction with their numerical values. Abbreviate the units as shown: cal, kcal, µg, mg, g (or gm), %, ^{o}C, nm, µm (not m), mm, cm, cm^3, m, in. (or write out inch), h (or hr), min, s (or sec), ml [write out liter(s)], kg. Wherever commonly used units are used their conversion factors must be shown at their first occurrence. Greek symbols are permitted as long as they show clearly in the soft copy.

- **References and notes** should be numbered in the order in which they are cited, starting with the text and then through the table and figure legends. Each reference should have a unique number and any references to unpublished data should be given a number in the text and referred to in the references. References should follow the standards presented in the AIC Reference Style Guidelines below.

REFERENCE STYLE GUIDELINES

References should be cited as numbers within square brackets [] at the appropriate place in the text. The reference numbers should be cited in the correct order throughout the text (including those in tables and figure captions, numbered according to where the table or figure is designated to appear). The references themselves are listed in numerical order at the end of the final printed text along with any Notes. Journal abbreviations should be consistent with those presented in Chemical Abstracts Service Source Index (CASSI) (http://www.cas.org) guide available at most academic libraries.

- **Names** and initials of all authors should always be given in the reference and must not be replaced by the phrase *et al*. This does not preclude one from referring to them by the first author, et al in the text.

- **Tables** should be in numerical order as they appear in the text and they should not duplicate the text. Tables should be completely understandable without reading the text. Every table should have a title. Table titles should be placed above the respective tables.

  Table 1. Bond Lengths (Å) of 2-aminophenol

- **Figure legends** should be in numerical order as they appear in the text. Legends should be limited to 250 words.

  Figure 1. PVC Melt Flow Characterized by Analytical Structural Method

- **Letters and Book Reviews** should be clearly indicated as such when being submitted. They are not peer-reviewed and are published as submitted. Legends should be placed after/under the respective figures.

- **Journals -** The general format for citations should be in the order: **author(s), journal, year, volume, page**. Page number ranges are preferred over single values, but either format is acceptable. Where page numbers are not yet known, articles may be cited by DOI (Digital Object Identifier). For example:

  Booth DE, Isenhour TL. *The Chemist*, 2000, 77(6), 7-14.

- **Books -** For example:

  Turner GK in *Chemiluminescence: Applications*, ed. Knox Van Dyke, CRC Press, Boca Raton, 1985, vol 1, ch. 3, pp 43-78.

- **Patents** should be indicated in the following form:

  McCapra F, Tutt D, Topping RM, UK Patent Number 1 461 877, 1973.

- **Reports and bulletins, etc. -** For example:

  Smith AB, Jones CD, *Environmental Impact Report for the US*, final report to the National Science Foundation on Grant AAA-999999, Any University, Philadelphia, PA, 2006.

- **Material presented at meetings -** For example:

  Smith AB. Presented at the Pittsburgh Conference, Atlantic City, NJ, March 1983, paper 101.

- **Theses -** For example:

  Jones AB, Ph.D. Thesis, Columbia University, 2004.

- For material presented at a meeting, congress or before a Society, etc., but not published, the following form should be used:

  Jones AB, presented in part at the 20th American Institute of Chemists National Meeting, Philadelphia, PA, June, 2004.

- For material accepted for publication, but not yet published, the following form should be used:

  Smith AB. *Anal. Chem*., in press

- For material submitted for publication but not yet accepted the following form should be used:

  Jones AB, *Anal. Chem*. submitted for publication.

- For personal communications the following should be used:

  Smith AB, personal communication.

- If material is to be published but has not yet been submitted the following form should be used:

  Smith AB, unpublished work.

Reference to unpublished work should not be made without the permission of those by whom the work was performed.

Manuscript Selection

The submission and review process is completely electronic. Submitted papers are assigned by the Editors, when appropriate, to at least two external reviewers anonymously. Reviewers will have approximately 10 days to submit their comments. In selected situations the review process can be expedited. Selected papers will be edited for clarity, accuracy, or to shorten, if necessary. The Editor-in-Chief will have final say over the acceptance of submissions. Most papers are published in the next issue after acceptance. Proofs will be sent to the corresponding author for review and approval. Authors will be charged for excessive alterations at the discretion of the Editor-in-Chief.

Conditions of Acceptance

When a paper is accepted by *The Chemist* for publication, it is understood that:

- Any reasonable request for materials to verify the conclusions or experiments will be honored.

- Authors retain copyright but agree to allow *The Chemist* to exclusive license to publish the submission in print or online.
- Authors agree to disclose all affiliations, funding sources, and financial or management relationships that could be perceived as potential conflicts of interest or biases.
- The submission will remain a privileged document and will not be released to the public or press before publication.
- The authors certify that all information described in their submission is original research reported for the first time within the submission and that the data and conclusions reported are correct and ethically obtained.
- The Chemist, the referees, and the AIC bear no responsibility for accuracy or validity of the submission.

Authorship

By submitting a manuscript, the corresponding author accepts the responsibility that all authors have agreed to be listed and have seen and approved of all aspects of the manuscript including its submission to *The Chemist*.

Submissions

Authors are required to submit their manuscripts, book reviews and letters electronically. They can be submitted via email at aicoffice@theaic.org with "Submission for consideration in *The Chemist*" in the subject line. All submissions should be in Microsoft® Word format.

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Announcements

INVITATION TO AUTHORS

Authors are invited to submit manuscripts for ***The Chemist,*** the official online refereed journal of The American Institute of Chemists (AIC). We accept submissions from all fields of chemistry defined broadly (e.g., scientific, educational, socio-political). *The Chemist* will not consider any paper or part of a paper that has been published or is under consideration for publication anywhere else.

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The Editor-in-Chief, The Chemist
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American Institute of Chemists

www.TheAIC.org

From its earliest days in 1923 to the present, the American Institute of Chemists has fostered the advancement of the chemical profession in the United States.

The Institute has a corresponding dedication "to promote and protect the public welfare; to establish and maintain standards of practice for these professions; and to promote the professional experience through certification as to encourage competent and efficient service."

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