5th Annual
Science Symposium
May 11, 1999
Paradise Valley Community College
Foreword

The 5th annual Science Symposium was held on May 11, 1999. Students enrolled in General Organic Chemistry II, CHM 236, participated in the event.

Each contributor was responsible for selecting and researching their topic, preparing a paper and orally presenting their project to their peers. This booklet contains each of those papers.

As an instructor and faculty advisor for this symposium, I want to thank and congratulate each participant for their effort, courage and dedication. By participating these individuals perpetuate this event annually. I am both proud and honored to present the work of these individuals.

I would also like to dedicate this symposium to my friend and colleague, Dr. Millard Lee. He is retiring from PVCC and without his leadership, guidance and friendship, I would have had difficulty with my transition to education from private industry. He is and will always be regarded as a wonderful teacher and friend.

William L. Mancini, PhD
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FLUOXETINE HYDROCHLORIDE

(PROZAC)

By : Nancy Andrade

April 21, 1999
Abstract: Fluoxetine Hydrochloride C\textsubscript{17} H\textsubscript{18} F\textsubscript{3} NO \textsuperscript{*} HCL most commonly known as Prozac, has been available in the United States since 1988, the first of a new class of anti-depressants known as Selective Serotonin Re-uptake Inhibitors (SSRIs)'s available in the United States. Children's prescriptions for Prozac-type drugs has risen by 80 percent in recent years. The disposition of single doses of fluoxetine in healthy elderly subjects (greater than 65 years of age) did not differ significantly from that in younger normal subjects.

Prozac

Prozac (Fluoxetine with Hydrochloride derivative) is an anti depressant designed for oral consumption. Its synonym is (+)-N-Methyl-3-Phenyl-3-[(alpha,alpha, alpha-Trifluoro-p-tolyl)oxy] Propylamine Hydrochloride. Its empirical formula is C\textsubscript{17} H\textsubscript{18} F\textsubscript{3} NO \textsuperscript{*} HCL, and its molecular weight is 345.79 g/mol. Fluoxetine has a melting point of 141-142 degrees Celsius with its derivative (HCL) of 139-140 degrees Celsius. Fluoxetine Hydrochloride is a white to off white crystalline solid with a solubility of 14 mg/ml in water. It is well absorbed by the body and food does not affect the extent of its absorption, although the absorption rate may be slightly decreased. The structural formula is: (1)

Eighty percent of the drug is excreted in the urine, and 15 percent in the feces, however it is not dialyzable because of high protein binding capacity 94.5 percent. (2) The half-life of Fluoxetine Hydrochloride after a single dose is two days (range 1 to 4 days) and after multiple dosing 4 days (range 2 to 7 days). The corresponding values for Norfluoxetine are similar after single and multiple dosing 8.6 and 9.3 days (range 4 to 15 days). After 30 days of dosing at 40 mg /day, plasma concentrations of Fluoxetine and Norfluoxetine ranged from 91 to
302 mg/ml and 72 to 258 mg/ml. Plasma concentrations of Fluoxetine were higher than those predicted from single dose studies presumably because Fluoxetine’s metabolism is not proportional to dose. Norfluoxetine, however, appears to have linear pharmacokinetics. Steady state plasma levels are attained after 4 to 5 weeks of continuous drug administration. Patients receiving Fluoxetine at doses of 40 to 80 mg/day over periods as long as three years exhibited on average, plasma concentrations similar to those seen among patients treated for 4 to 5 weeks. Similarly, because of the long half-lives of Fluoxetine and Norfluoxetine, it may take up to 1 to 2 months for the active drug substance to disappear from the body. (3)

The oral solution contains Fluoxetine Hydrochloride equivalent to 20 mg/5ml of Fluoxetine. It also contains 23 percent benzoic acid, flavoring agent, glycerin, purified water, and sucrose.

Clinical Pharmacology

Studies in animals suggest that Fluoxetine is a much more potent uptake inhibitor of Serotonin than Norepinephrine.

Systemic Bioavailability

In man, following a single oral 40 mg dose, peak plasma concentrations of Fluoxetine from 15 to 55 mg/ml are observed after 6 to 8 hours. The disposition of single doses of Fluoxetine in healthy elderly subjects (greater than 65 years of age) did not differ significantly from that in younger normal subjects.

Clinical experience with Prozac in patients with concomitant systemic illness is limited. Caution is advisable when using Prozac in patients with diseases or conditions that could affect metabolism or hemodynamic responses. Fluoxetine has not been evaluated or used to any appreciable extent in patients with a recent history of myocardial infarction or unstable heart disease. Patients with these diagnoses were systematically excluded from the clinical studies during the product’s pre-market testing. However, the electrocardiograms of 312 patients who received Prozac were evaluated and no conduction abnormalities that resulted in heart block were observed. The mean heart rate was reduced by approximately 3 beats/minute.
In patients with diabetes, Prozac may alter glycemic control. Hypoglycemia has occurred during therapy with Prozac, and hyperglycemia has developed following discontinuation of the drug. As is true with many other types of medication when taken concurrently by patients with diabetes, insulin and or oral hypoglycemic dosage may need to be adjusted when therapy with Prozac is instituted or discontinued.

For nursing mothers, because Prozac is excreted in human milk nursing while on Prozac is not recommended. In a breast milk sample, the concentration of Fluoxetine plus Norfluoxetine was 70.4 mg/ml. No adverse effects in the infant were reported. In another case, an infant’s plasma drug levels were 340 mg/ml of Fluoxetine, and 208 mg/ml of Fluoxetine on second day of feeding.

Fluoxetine Hydrochloride is not only used to treat patients with depression, but bulimia nervosa by significantly decreasing binge-eating and purging activity when compared with placebo treatment. Also obsessive-compulsive disorder has been known to be treated with Prozac. Prozac in this case reduces symptoms of the disease.

The chemistry of Prozac

Fluoxetine is a racemic mixture (50/50) of R-Fluoxetine and S-Fluoxetine enantiomers. In animal models, both enantiomers are specific and potent Serotonin uptake inhibitors with essentially equivalent pharmacologic activity. The S-Fluoxetine enantiomers is eliminated more slowly and is the predominant enantiomer present in plasma at steady state.

Prozac or Fluoxetine, belongs to a general group of compounds called Phenoxyphenyl Propylamines. Studying the Ki derivatives of Fluoxetine, it has demonstrated its potency as an inhibitor of Serotonin (5-HT, or 5-Hydroxytryptamine). Table 1 indicates the substituents and their resulting Ki. (4)
Table 1: Inhibition of 5-HT (Serotonin) by Phenoxyphenylpropylamines

<table>
<thead>
<tr>
<th>Substituent</th>
<th>Amine Substituent</th>
<th>Phenoxy Ring Substituent</th>
<th>Phenyl Ring Substituent</th>
<th>Inhibition</th>
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<tr>
<td>H</td>
<td>CH₃</td>
<td>H</td>
<td>H</td>
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<td>H</td>
<td>CH₃</td>
<td>o-CF₃</td>
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<td>m-CF₃</td>
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</tr>
<tr>
<td>H (Fluoxetine)</td>
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<td>CH₃</td>
<td>p-OCH₃</td>
<td>H</td>
<td>.07</td>
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</table>

Wong and Bymaster found five characteristics of Phenoxyphenylpropylamines that either contributed or did not contribute to their inhibition of Serotonin uptake in the brain. These included:

1. Alkylamine side chain
   Researchers found that the most effective inhibitor of Serotonin uptake had an Alkylamine side chain consisting of 3 carbons.

2. A phenyl ring attached to the carbon with the phenoxy ring
   Adding a phenyl ring to this carbon, which is also connected to the phenoxy ring, enhanced the potency of Serotonin inhibition almost 41-fold.

3. The position of the substituent on the phenoxy ring
   Compare the Ki of the para-CF₃ which has significantly larger Kᵢ's than that of the para-substitution. From this information, we can guess that a derivative which has the large -CF₃ group so close to the oxygen of the phenoxy group has a higher-energy conformation and thus is not as likely to be as stable as the meta- or ortho-derivatives at inhibiting Serotonin uptake.

4. The identity of the para-substituent on the Phenoxy ring
The derivative with a para-substitution of CF₃ has a smaller Ki than derivatives with para-substituents of hydrogen, fluorine, chlorine, -CH₃ (methyl) or -OCH₃ (methoxy) groups. Note that it is probably the size of the CF₃ group that is important, because it has a larger molecular weight than any of the other substituents.

5. Substitution of the primary amine group
The substitution of the primary amine group does not affect the ability of the derivative to inhibit Serotonin uptake. This is shown by the fact that Fluoxetine, which has a methyl and hydroxyl substitution on the amine, has the same Ki (0.02) as Norfluoxetine, which has two hydroxyl substituents on the amine.

The Synthesis of R- and S- Fluoxetine

(5) 3 Phenyl-3-hydroxypropylamine was created by starting with (3) R-styrene oxide reduction with Acetone Cyanohydrin with Triethylamine gave (4) S-3-Phenyl-3-hydroxy propanenitrile. Reduction of (4) with Burane-methyl sulfide complex provided (5) 3 Phenyl-3-hidroxy propylamine. This intermediate was then used to create Fluoxetine. (5)
(5) was reacted with NaH/DMSU, 4-ClC₆H₄CF₃, HCl to produce (6) Norfluoxetine Hydrochloride. In order to make (8) Fluoxetine Hydrochloride, CICO₂Me, followed by LAH to make Norfluoxetine Hydrochloride. 90 percent yield was obtained. This approach compliments other existing methods of creating Fluoxetine Hydrochloride, but this method takes advantage of enatiomerically pure starting material unlike other methods.

Psychological effects of Prozac

In a personal interview with a 24 year old female student, working full time and who is also a full time student, she confessed to have benefited from the effects of Prozac at 21 years of age. She revealed that she had many psychological problems going on in her life at that time, some of which were considered to be severe symptoms of depression. Even though she knew something was wrong with her, little did she know she was suffering from a high level of depression. She was suffering from lack of concentration, sleeplessness at work and at school. She had no energy to do anything, even the things she enjoyed doing, she cried at night, and was constantly sad. She remembers developing a negative way of thinking, and worse of all, suicidal thoughts.

She was taking a psychology course at that time, and as a coincidence she found out she was ill by answering a questionnaire for depression. This combined with curiosity concerned go to see a counselor. Later, a physician prescribed Prozac as the best alternative. Prozac combined with counseling at the same time, helped her.

Prozac saved her life and brought her life back together. She did not have any side effects; she was on Prozac for two months. She is now pregnant, about to give birth, and about to get married. She is glad she found help when she most needed it, and recommends anyone who needs it to use it. (6)

The alteration of brain chemistry experienced during depression is thought to involve the serotogenic synapse. Serotonin, or 5-HT, or 5-Hydroxytryptamine, is a neurotransmitter with its structure shown below.
It is thought that low level of Serotonin at post-synaptic receptors causes depression. This theory is supported by the fact that concentrations of Serotonin are lower in the brains of those killed in suicide than those killed in sudden death. (7)

![Chemical structure of Serotonin, 5-HT](image)

Serotonin, 5-HT

The advantage of Prozac over other antidepressant drugs such as the tricyclic amines (TCA's), such as (Elavil-Amitryptilamine) is that Prozac, as with all selective Serotonin re-uptake inhibitors, has low binding for other receptors. This has proved beneficial because the TCA's show binding to musarinic, and histamine receptors that opens the door to an abundance of side effects that can be avoided when taking Prozac.

**Action of 5-HT**

![Diagram of serotonin action](image)

Figure 1 depicts the general scheme of how a serotonergic neuron works. The important steps related to Prozac are steps 3, where Serotonin is released into the synapse; step 4 where Serotonin binds to the post-synaptic receptors; and step 5 where Serotonin is reabsorbed into the presynaptic neuron in a process called re-uptake. Re-
uptake is the mechanism that prevents overstimulation of the postsynaptic cell.

Do Children Need Prozac?

In today’s hectic society with its many pressures more children are being diagnosed with depression. Dr. Lawrence Diller, a behavioral pediatrician in Walnut Creek, California, suspects that some parents are trying to cure their children of anguish that is simply part of growing up. Others worry that children are receiving anti-depressants not as an adjunct to care and counseling, but as a cheap substitute. Depression in children is often the long-term illness that the child and family will have to deal with, says Dr. Anne McBride, a child psychiatrist at New York Hospital-Cornell Medical Center. A pill alone is not usually the cure. Support and counseling of the family are important parts of the treatment. When the cost-cutting pressure is on, doctors may think that all they can do is prescribe. (8)

FDA concerned about rising use of medication among youth

Dr. Harold Koplewicks, director of the Child Study Center at New York University Medical Center, said “The most important thing is making sure the child is correctly diagnosed. Today’s debate is not about whether children can be depressed, but about the best way to treat them. Drugs therapy or both? “

President Clinton recently announced a plan that would require drug companies to test whether medicines are safe and effective for children.

FDA officials also are concerned that the use of anti-depressants in children is on the rise with little information available about their use. The agencies are urging drug companies to begin studies.

Also, the National Institute of mental health has just supported the opening of three child psychopharmacology centers at Columbia
University's College of physicians and surgeons, Johns Hopkins Medical Institutes, and the University of Pittsburgh. These centers will conduct medication studies on children with depression and anxiety disorders.

Dr. Peter Jensen, Chief of NIMH's child and adolescent research branch, said fewer than 500 children have participated in trials, and most of the studies were too small to show differences between drugs and placebos.

Still in 1994, 200,000 prescriptions for Prozac and 300,000 for Zoloft were filled for children from five to ten years of age. Another 150,000 prescriptions for Zoloft and an equal amount for Prozac were filled for adolescents.

The popular medicine's makers Eli Lilly and company will use the data to petition the FDA to use the drug in children in adolescents. So far, no drugs have been approved for depression in people under 18. (9)

Prozac (Fluoxetine Hydrochloride) is a drug that has been widely used in the United States. People from 5 to over 65 years of age have benefited from Prozac. There are other existing methods to synthesize Prozac (Fluoxetine Hydrochloride), but the best method is to use the intermediate, 3-phenyl-3-Hydroxypropylamine, because it takes advantage of enantiomerically pure starting material unlike other existing methods.
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DIGOXIN

BY: ROBBY ARANKI
04/23/99
Abstract

The drug Digoxin (Lanoxin) has a long history as an effective treatment of mild to moderate heart failure. It has been found to be useful in helping people live longer and healthier. Digoxin is a naturally occurring substance found in the foxglove plant that has dropped the mortality rates in long term congestive heart failure patients. This paper will discuss the chemistry, structure, synthesis, and clinical uses of Digoxin.

Digoxin is one of the cardiac glycosides, a closely related group of drugs having common effects on the myocardium. It is commonly known as digitalis, Lanoxin, and Lanoxin caps (brand names). These drugs are found in a number of plants. Digoxin is extracted from the leaves of Digitalis lanata. The ancient Egyptians used cardiac glycosides as sources of arrow poisons, and the Romans used them as a cardiovascular tonic. Digoxin is indicated for the treatment of congestive heart failure and to control ventricular rate in the treatment of chronic atrial fibrillation and atrial flutter. While digoxin increases left ventricular ejection fractions, improves symptoms, and reduces the need for hospitalization in heart failure patients, overall mortality is effected (1).

Although digoxin has been used for decades in patients with heart failure, ACE inhibitors have helped digoxin as first line therapy for congestive heart failure due to systolic dysfunction(2). In patients with atrial fibrillation or atrial flutter, calcium-channel blockers, such as verapamil and diltiazem, are generally more effective than digoxin for controlling ventricular rate. Although digoxin is used for the treatment and/or prophylaxis of supraventricular arrhythmias due to reentry mechanisms, calcium antagonist are usually preferred. But, still digoxin is a rapid acting cardiac glycoside that has both direct and indirect effects(3). The direct effects include increased force and velocity of myocardial systolic contraction, increased refractory period of the AV node, and increased total peripheral resistance. The first commercially available digoxin products approved by the FDA went on the market in 1952. Here is an Ultraviolet Spectrum Absorption of Digoxin:

FIGURE 2.4. Ultraviolet Absorbance Spectrum of Digoxin

The ultraviolet absorbance spectrum of a 40 mg/L solution of digoxin from Burroughs Wellcome, Lot No. 61016, in 80% ethanol (v/v) was obtained with a Cary Model 118 Spectrophotometer and 1-cm matched quartz cells. The reference cell contained 80% ethanol.
Two 12 week, double blind, placebo controlled trials enrolled a total of 266 patients with New York Heart Association (NYHA) class II or III heart failure, previously treated with Digoxin, a diuretic and an angiotensin converting enzyme (ACE) inhibitor. Patients were randomized to placebo or Lanoxin groups. Both trials demonstrated better preservation of exercise capacity in the Lanoxin treated group. Continued treatment with Lanoxin reduced the risk of developing worsening heart failure as measured by rehospitalization rates, need for emergency care and need for concomitant heart failure therapy.

In a larger study of 6,081 patients the use of Lanoxin was associated with a trend in reduction in time to all cause death or hospitalization. The trend was evident in subgroups of patients with mild heart failure as well as with more severe disease. In patients with chronic atrial fibrillation, digoxin slows rapid ventricular response rate in a linear dose response fashion from 0.25 to 0.75 mg/day.

Digoxin molecules are comprised of two portions, a sugar unit and a cardenolide(4). Digoxin has the molecular formula $C_{41}H_{64}O_{14}$, a molecular weight of 780.95 grams and a melting and decomposition points above 235 degrees Celsius. The drug is practically insoluble in water and in ether, it is slightly soluble in diluted 50 percent alcohol and in chloroform; and freely soluble in pyridine. Digoxin powder is composed of odorless white crystals(5).

Digoxin has the chemical name:
3beta-[(3)-0-2,6-dideoxy-beta-D-ribo-hexopyranosyl-(1->4)-0-2,6-dideoxy-beta-D-ribo-hexopyranosyl-(1->4)-2,6-dideoxy-beta-D-ribo-hexopyranosyl]oxy]-12beta, 14-dihydroxy-5beta-card-20(22)-enolide, and the structure shown:
Digoxin inhibits the Na-K-ATPase membrane pump. Na-K-ATPase regulate intracellular sodium and potassium. Inhibitors of this enzyme leads to an increase in intracellular sodium concentration (i.e., decreased outward transport) and ultimately to an increase in intracellular calcium(6). End-dialostic pressures decrease, leading to a reduction in pulmonary and systemic venous pressures. In patients with normal hearts, however, cardiac output remains unchanged.

Digoxin also possesses direct vasoconstrictive properties and reflex CNS-mediated peripheral vasoconstriction(7). Although this increases vascular resistance, in patients with failing hearts, increased myocardial contractility predominates and total peripheral resistance drops. In patients with congestive heart failure, an increased cardiac output will decrease sympathetic tone, thereby reducing the heart rate and causing diuresis in edematous patients and improving coronary blood flow.

In addition to its inotropic effects, digoxin also possesses significant actions on the electrical activity of the heart. It increases the slope phase 4 depolarization, shortens the action potential duration, and decreases the maximal diastolic potential(8). The increase in vagal activity mediated by cardiac glycosides decreases conduction velocity through the atrioventricular (AV) node, prolonging its effective refractory period.

In atrial flutter of fibrillation, digoxin decreases the number of atrial depolarizations that reach the ventricle, thereby slowing ventricular rate(9). Sympathetic stimulation, however, easily override the beneficial inhibitory effects of digoxin on AV nodal conduction. Thus, verapamil and diltiazem are gradually replacing digoxin as the agent to control ventricular rate in atrial tachyarrhythmias. While digoxin is somewhat effective in controlling ventricular rate in atrial fibrillation, it appears to be no better than placebo for converting recent-onset atrial fibrillation to normal sinus rhythm.

Digoxin is commercially available as tablets, capsules, oral elixir, and injection in general, digoxin is rapidly absorbed from the GI tract following oral does. Bioavailability from capsules is essentially complete but is approximately 75-85% from the oral elixir and 70-80% from tablets. Digoxin distributes throughout the body tissues, with the highest concentrations found in the heart, kidneys, intestine, liver, stomach and skeletal muscle(10). Small amounts can be found in the brain. The presence of congestive heart failure slows the rate at which steady-state distribution is achieved. Only 20-30% of the drug is plasma protein-bound. Digoxin crosses the placenta, and maternal and fetal plasma concentrations of the drug are equal. Onset of therapeutic effects generally occurs within 30 minutes to 2 hours after oral administration and within 5-30 minutes following IV administration. The peak effect generally occurs between 2-6 hours after oral administration of a dose.
A small amount of digoxin is metabolized in the liver to inactivate metabolites(11). In approximately 10% of patients, however, significant amounts of orally ingested digoxin are metabolized in the gut by intestinal bacteria and about 20-30% of digoxin in blood is bound to plasma proteins (albumins). Thirty to fifty percent of a dose is excreted unchanged in the urine. The elimination half-life of digoxin in adults is normally 30-40 hours, but heart failure or renal impairment can prolong digoxin elimination. Thus, in patients with renal impairment, the half-life in infants and full-term neonates is 18-25 hours and 35-45 hours, respectively; digoxin half-life is prolonged in premature neonates is (e.g., 61-170 hours).

Potassium depleting diuretics and corticosteroids are a major contributing factor to digitalis toxicity. Calcium, particularly if administered rapidly by the intravenous route may produce serious arrhythmias in digitalized patients. Quinidine, verapamil, amiodarone, and propafenone cause a rise in serum digoxin concentration, with the implication that digitalis intoxication may result.

Certain antibiotics increase digoxin absorption in patients who inactivate digoxin by bacterial metabolism in the lower intestine, so that digitalis intoxication may result. Propantheline and diphenoxylate, by decreasing gut motility, may increase digoxin absorption. Antacids, kaolin-pectin, and certain anticancer drugs may interfere with intestinal digoxin absorption resulting in unexpectable low serum concentrations. The following table list drugs which may effect serum levels of digoxin:
### Drugs Affecting Serum Levels of Digoxin

<table>
<thead>
<tr>
<th>Drugs Which May Increase Serum Levels of Digoxin</th>
<th>Drugs Which May Decrease Serum Levels of Digoxin</th>
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<tbody>
<tr>
<td>Verapamil</td>
<td>Antacids</td>
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<tr>
<td>Tetracycline</td>
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<td>Rifampin</td>
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<tr>
<td>Diphenoxylate</td>
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</table>

In general, adverse reactions of digoxin are dose-dependent and occur at doses higher than those needed to achieve a therapeutic effect. Hence, adverse events are less common when digoxin is administered at the recommended dose range or maintained within recommended serum concentration range. Some patients may be particularly susceptible to digoxin toxicity and the drug dosage should always be selected carefully and adjusted as the clinical situation warrants.

Manifestations of life-threatening toxicity include severe ventricular arrhythmias such as ventricular tachycardia or ventricular fibrillation, or progressive bradyarrhythmias such as severe as sinus bradycardia or second or third degree heart block not responsive to atropine. An overdosage of more than 10 mg of digoxin in previously healthy adults or 4 mg in previously healthy children or overdosage resulting in steady-state serum concentrations greater than 10 ng/ml, often results in cardiac arrest.

Severe digitalis intoxication can cause life-threatening elevation in serum potassium concentration by shifting potassium from inside to outside the cell resulting in hyperkalemia. Administration of potassium supplements in the setting of massive intoxication may be hazardous. Signs and symptoms of digitalis toxicity include:

1. G.I. distress (anorexia, nausea, vomiting, and diarrhea for more than 1 day).
3. Weakness.
4. Fatigue.
5. S.T. segment sagging and shortened Q wave are normal findings for digitalized
patients.
6. Suspect digitalis toxicity if the following E.C.G. rhythms are observed;
A. Atrial Tachycardia.
B. Atrial tachycardia with block.
C. Junctional tachycardia.
D. Bi-directional ventricular tachycardia.
E. Bradycardiac atrial fibrillation.

Patients with massive digitalis ingestion should receive large doses of activated charcoal to prevent absorption and bind digoxin in the gut during enteroenteric recirculation. Emesis or gastric lavage may be indicated especially if ingestion has occurred within 30 minutes of the patient’s presentation at the hospital. Emesis should not be induced in patients who are obtunded.

If a patient presents more than 2 hours after ingestion or already has toxic manifestations, it may be unsafe to induce vomiting or attempt passage of a gastric tube, because such maneuvers may induce an acute vagal episode that can worsen digitalis-toxic arrhythmias.

Digoxin immune Fab is an antigen-binding agent which is used as an antidote. The drug is a sterile preparation of monovalent, digoxin-specific antigen binding fragments Fab) derived from antidigoxin antibodies. The antidigoxin antibodies are obtained from the serum of healthy sheep that have been immunized with a conjugate of digoxin (the hapten) and albumin human (a protein carrier). The antidigoxin antibodies are then cleaved by papain to form a Fc fragment (crystallizable fragment that contains most of the antigenic and complement-binding determinants) and 2 identical monovalent Fab fragments. Following enzymatic cleavage, digoxin-specific Fab is isolated and purified via affinity chromatography, yielding fragments with a molecular mass of approximately 46,200 daltons.

Digoxin obviously has a long line of followers and its number of users continues to grow without signs of stopping. Will Digoxin supplementation continue to be a safe choice for helping congestive heart failure patients (CHF)? Much research still remains to be done on Digoxin, but however looking at the synthesis and structure activity relationships of the drug, other compounds are being developed for possible use and reinforcement. For example, people including my younger brother who have had CHF and were prescribed Digoxin found that it really works. Time will tell.
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Barbituric Acid
Derivatives and uses

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Organic Chemistry 236
April 23, 1999
Abstract: Barbituric acid is a purely synthetic organic compound, not found in nature, which has had significant impact in heterocyclic chemistry and in the way we live. Unlike many of our heterocyclic compounds which are formed via raw materials produced by biological methods, Barbituric acid and its derivatives form numerous psychologically active pharmaceuticals and various polymers used in the plastics industry. So diverse are its applications, it can easily be considered to have the personality of a conventional alcohol. This paper is dedicated to the study of its impact, both as an academic understanding of its reactions, as well as its applications in our lives.

The discovery of Barbituric acid dates back to 1864, when it was first synthesized by the famous chemist, Adolph von Baeyer, by reacting diethyl malonate with urea in the presence of a sodium ethoxide reagent (see figure A). The name Barbituric acid is a combination of the name of an "anonymous" Barbara and the raw materials of ethyl malonate and urea that were utilized by Von Baeyer to synthesize the heterocyclic ureide, malonylurea.

(Figure A)

\[
\text{Urea} + \text{Ethyl malonate} \xrightarrow{\text{NaOCH}_2\text{C}_2\text{H}_5, \text{CH}_3\text{OH}, 110^\circ\text{C}} \text{Barbituric acid (Malonylurea)}
\]

Barbituric acid became the "mother compound" to the class of drugs that we now know today as barbiturates (classified by the USDA as "controlled substances"). Barbiturates are chemically related derivatives of barbituric acid characterized by their depressant effect on the central nervous system (CNS). They are utilized primarily as tranquilizers and sophorics (sleep inducers). Most barbiturates exert a sedative effect in low doses and hypnotic effect in high doses. Barbituric acid, itself, does not exert the hypnotic effect that its derivatives are known for.

The first barbiturate to be used clinically was Veronal* (also called Barbital - see figure B) in 1903. Phenobarbital, now most commonly used to counteract seizures, was introduced in 1912. Over 2,500 barbiturates have been synthesized since then, and at the height of their popularity about 50 were marketed for human use; until the 1960's when Benzodiazepines replaced them as the drug of choice when antianxiety, sedative, hypnotic or anticonvulsant action is required. Today only about a dozen are used.
Veronal was named after the city of Verona, because the chemist that it was synthesized under felt it was "The most restful city on earth", thus referencing its hypnotic effects.

**Physical and Chemical Properties:** Barbituric acid, also known as Malonylurea or 2,4,6-Pyrimidinetrione has a molecular formula $\text{C}_4\text{H}_4\text{N}_2\text{O}_3$, and its molecular weight is 128.087. It has a melting point of 248 degrees Celsius. Physically, it is seen in the form of a white, powdery solid (dihydrate prisms). It is considered an acid because the carbonyl groups render the imide hydrogens acidic (see below).

The structural formula is:
Tautomeric structures:

Structures of some pharmaceutical derivatives:

Sodium pentothal
Amobarbital
Pentobarbital
Phenobarbital

The following is an Infra-red spectrum for Barbituric acid: Note the peaks.
The following is an NMR spectrum analysis of Barbituric acid: (1H, 3H, 5H)

Chemical Synthesis: Barbituric acid is synthesized by the treatment of urea with acid chlorides or anhydrides to create ureides.

\[
\begin{align*}
H_2N-C-NH_2 + CH_3COC\! &=\! CH_2C\!=\!O \\
\xrightarrow{\text{Acetylene}} \quad \text{Acetylene} \\
\text{A ureide}
\end{align*}
\]

Urea, combined with Ethyl malonate and reacted with sodium ethoxide, yields barbituric acid.

Synthesis of Phenobarbital:
**Pharmacology:** All barbiturates are weak acid, and their salts are well-absorbed both orally and intramuscularly. Barbiturates are usually classified by the duration of their clinical action, which depends on the rate of absorption, lipid solubility, serum binding, and mode of metabolism. Most barbiturates undergo side chain oxidation by liver microsomal enzymes. The resulting hydroxylated metabolites are excreted in the urine as glucuronide conjugates. Specificity of the liver microsomal enzymes for the individual barbiturates is a major factor in determining their duration of action. Phenobarbital, used primarily in the treatment of seizure disorders, is a long-acting barbiturate with a half-life of 2-6 days. It is poorly metabolized, and approximately 20% of the drug is excreted unchanged in the urine. Meprobamate is metabolized by N-demethylation in the liver to Phenobarbital. The short-acting barbiturates, which include phenobarbital, amobarbital, and secocholine, are used as hypnotics and presurgical medication. These drugs have half-lives of 20-40 hours. The ultrashort-acting barbiturates thiopental, methohexital, and thiamylal are used as intravenous general anesthetics and have a half-life of less than 8 hours. Only traces of short-acting barbiturates are excreted unchanged in the urine.

**Toxicity:** Barbiturates have a profound depressant effect on the central nervous system, and symptoms of toxicity may progress from apparent alcoholic intoxication through sedation, coma with reflexes present, deep coma, and eventually to circulatory and respiratory collapse. Severe poisoning is likely to occur when more than ten times the hypnotic dose is ingested at one time. Fifteen to twenty times the hypnotic dose may result in death. Barbiturates are central nervous system (CNS) depressants which are derivatives of barbituric acid. Barbituric acid, which has not CNS depressant activity itself, was first synthesized in the last century by the condensation of urea and Masonic acid. The active derivatives all have alkyl or aryl groups replacing the hydrogen atoms attached at the 5-carbon position. The first such compound, diethylbarbituric acid, was synthesized in 1903 and marketed as barbital (Vernal). The second Phenobarbital (Luminal), was an ethyl and a phenyl group substituted, was introduced in 1922. Phenobarbital is still widely used, not so much as a sedative but as an anticonvulsant.

**Lipid solubility:** Both Barbital and Phenobarbital proved to be long acting. Structural changes which increase the solubility in lipids shortened the duration of action and increased potency. Related compounds in which the 2-carbon (that derived from urea) is replaced by a sulfur atom are even more lipid-soluble and have a very short duration of action. Although these compounds are not, strictly speaking, barbiturates, they are generally regarded as such and often referred to as thiobarbiturates.

The barbiturates are usually classified clinically according to their duration of action: the long-acting compounds such as Phenobarbital have biologic half-lives of 80-120 hours; the intermediate group (e.g., butabarbitral) 30-50 hours, and the short-acting (secocholine) 15-20 hours. There is only an approximate relationship between the duration of action and the elimination of the drug from plasma. The expected duration of action for the three groups is 12, 6, and 3 hours, respectively.
Metabolism: To a large extent, the metabolism of barbiturates depends upon the degree of binding to plasma proteins. Those compounds such as phenobarbital, which are relatively polar, have low lipid-water partition coefficients, and are not bound to protein. They can pass though the kidney to be excreted into the urine unchanged. Alkalization promotes ionization of barbiturates and increases their urinary excretion. The non-polar barbiturates are protein-bound, so they do not appear in the urine.

That portion of the barbiturates which is not excreted unchanged in the urine is metabolized in the liver to inactive products. This includes most of the short-acting barbiturates. The biotransformation that involves oxidation of the radicals at the C-5 site to alcohols, ketones, carboxylic acids, phenols. These compounds may be excreted into the urine as such or conjugated with glucuronic acid.

Mechanism Of Action: Low doses of barbiturates are thought to enhance the inhibitory effects of gamma-aminobutyric acid (GABA, the natural inhibitor of nervous processes in the mammalian brain) on neural transmission. This effect, in benzodiazepines is similar, though the inhibitory action of GABA is less potent, making barbiturates more potent and therefore more hazardous.

Anticonvulsant /Clinical Uses: Barbiturates once were the major drugs employed as sedatives and hypnotics, but they have been displaced by the much safer benzodiazepines. Phenobarbital is still widely used as an anticonvulsant and has a few special uses, such as in the treatment of hyperbilirubinemia of the newborn, and reduction of oxygen consumption by the brain in patients with severe brain damage. The thiobarburate, thiopental, is commonly used in the induction of general anesthesia. Veterinarians still use barbiturates, more widely, in their anesthesia and euthanasia procedures.

Toxicity: In spite of the switch by the medical profession from barbiturates to benzodiazepines, vast quantities of barbiturates are still manufactured and are readily available in the United States. Barbiturate poisoning is still a common clinical problem. Most cases represent suicide attempts, but accidental ingestion by children and accidental drug overdosage by drug-abusers also contribute to approximately 40% of hospital emergency room visits.

The lethal dose varies greatly from drug to drug. Generally, the shorter-acting compounds are the most potent. A potentially fatal dose of secobarbital may be as low as 2 grams, whereas phenobarbital is unlike to cause death at doses lower than 8 grams. The prognostic significance of the plasma concentrations also varies with the relative lipid solubility. Death from pentobarbital has been reported at plasma concentrations of only 1 mg/dL, while to lowest reported lethal concentration of phenobarbital is 6 mg/dL.
The clinical manifestations of barbiturate intoxication are the same, regardless of which barbiturate is involved; only the rapidity of onset and duration of action differ. The signs and symptoms reflect depression of the CNS and the cardiovascular system. The subject’s behavior resembles alcohol intoxication. This proceeds to coma. Pulmonary ventilation is decreased. Unless compensated by artificial ventilation, this leads to cerebral hypoxia and further brain damage. The blood pressure falls due to hypoxia of the vascular centers in the medulla as well as a direct effect on the heart. Body metabolism is slowed and the body temperature falls. Pneumonia and kidney failure are common complications of barbiturate overdosage, and, if death ensues, are frequently the immediate cause.

**Environmental use:** Cyanide detection - Cyanide is used in many chemical and refining processes. It is found in the waste products from many manufacturing plants, including: electroplating and metal cleaning operations, coke ovens and steel manufacturing facilities. Although cyanide can be safely removed by alkaline chlorination, its acute toxicity to aquatic life necessitates routine monitoring of effluents prior to discharge. A limit of 0.01 mg/l cyanide in drinking water has been established.

The colorimetric method is based on the isonicotinic / barbituric acid procedure. Chlorine is added to the sample which has been buffered pH 6. The resulting cyanogen chloride reacts with isonicotinic and barburturic acids to form a blue color. Results are expressed as (mg/L) CN.

**Industrial use:** Polymer plastics and related products, which are beyond the scope of this paper.

**Stereochemistry:** Barbituric acid does not contain a chiral carbon and is not considered an optically active compound. However, some derivatives have carbon positioning which make for the possibility of optically active compounds.

**Practical synthesis in the laboratory:** The emphasis in working with Barbituric acid is that all portions of the experiment must be completely DRY. The use of sodium metal is required to make the sodium ethoxide. This requires, of course; anhydrous alcohol, due to the fact that any water would produce sodium hydroxide, which would interfere with the reaction. Water will destroy sodium ethoxide, creating sodium hydroxide and ethyl alcohol. Water can also be sourced as a contaminant from moisture in the air. The best-case scenario for this reaction is to blanket it with an inert anhydrous gas such as dry nitrogen.

The reaction is basically prepared by using all efforts to eliminate water. 100% ethanol, commercially available, may still contain minute traces of water. Distillation with
benzene, effecting a triazeotrope (or other methods of dehydration) is recommended for purification at the point of usage. The “absolute” ethanol is then reacted with sodium metal to produce sodium ethoxide. In the same reaction flask, add diethyl malonate and urea. Reflux for 2 hours. Observe a precipitate and acidify. Purify the product by cooling and recrystallization. This will produce white, powdered crystals.

Conclusion: Since their inception at the turn of the century, the barbiturates have provided a wealth of useful chemicals, as well as pharmaceuticals. Their unusual cyclic chemistry is currently mimicked in safer hypnotics, such as the Benzodiazepines. But, amazingly, its usefulness has shifted to the anticonvulsants. In polymeric and environmental applications barbituric acid lends itself to easy production, making it a commercially attractive substance. Additionally, the physiological applications of the barbiturates has yet to be exhausted. As current science / medicine has learned to roll back its older drugs to current uses, it is likely that those 2,500 known derivatives of Barbituric acid will again seek usage in our daily ways of living.
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Drug screen tests: Barbiturate, Benzodiazipine and Tricyclic Antidepressant screens

Cyanide. *Cyanide (free)*. cyanide.html at www.chemetrics.com
BE STRESS FREE WITH BZs

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APRIL 22, 1999
Abstract

Benzodiazepines are a large class of compounds that are used as sedatives, skeletal muscle relaxants and anti-anxiety agents. The compounds in this class have structures that are similar, but differ in the ways in which they are metabolized by the body. All benzodiazepines work in the same area of the central nervous system and produce the same relaxing effects. Some are more suited to a specific role because of differences in their chemical makeup that give them greater activity in a certain area or characteristics that make them more desirable for a certain function. The use of these drugs has skyrocketed in the search for relief from anxiety; benzodiazepines can be beneficial, but there are some risks to be aware of before taking any drug in this class.

Benzodiazepines (BZs) are known as minor tranquilizers or central nervous system (CNS) depressants. The first synthesized BZ, developed in 1957, was Librium (chlorodiazepoxide). It was discovered by accident by L. Sternbach and E. Reeder. They submitted a sample of what they thought could be an antibiotic, but determined the structure had rearranged itself during the synthesis to what is now known as Librium. The benzodiazepine that came shortly after Librium was Diazepam, commonly known as Valium. The approval of Valium by the FDA (Food and Drug Administration) occurred in 1963. After these discoveries many other benzodiazepines were developed.

There are over 20 commonly used benzodiazepines on the market today. Valium is a typical benzodiazepine and can be synthesized with relative ease with an overall yield of about 50% from the commercially available starting material 5-chloroisatoic anhydride.

5-Chloro-Isatoic Anhydride is Methylated with Methyl Iodide to the N-methyl compound
The Anhydride is rearranged with glycine to form 7-Chloro-1-methyl-3,4-dihydro-1H-1,4-benzodiazepine-2,5-dione

The benzodiazepine above is acetylated to 4-Acetyl-7-chloro-1-methyl-3,4-dihydro-1H-1,4-benzodiazepine-2,5-dione

This benzodiazepine is reacted with chlorobenzene Grignard to form 5-Chloro-2-((glycylmethylamino)-benzophenone which can be directly cyclized to Diazepam, but in lower yield than the oxime route below.

The above benzophenone is reacted with hydroxylamine to its oxime
The oxime is finally cyclized to form Diazepam, or 7-Chloro-1-methyl-5-phenyl-3H-1,4-benzodiazepin-2(1H-one).

Changing the position of substituents, increasing or decreasing the amount of nitrogen and/or oxygen, or by adding or removing ketones from the parent compound easily developed new benzodiazepines. In this process a wide variety of drugs with similar actions grew into an enormous class of drugs now known as benzodiazepines. In 1981 the FDA had approved two other BZs to add to the already large group, Xanax (Alprazolam) and Restoril (Temazepam). 2-3 The structures and empirical formulas between these three is very similar.

Diazepam 4 \( \text{C}_{16}\text{H}_{13}\text{Cl } \text{N}_2\text{O} \)

Alprazolam \( \text{C}_{17}\text{H}_{13}\text{Cl } \text{N}_4 \)
Temazepam  $\text{C}_{16}\text{H}_{13}\text{Cl N}_2\text{O}_2$

The chemical similarity among these formulas is an indication that they all work in the same region of the brain. All BZs work at the level of the limbic, thalamic and hypothalamic regions of the central nervous system.$^5$ "The action of these drugs is mediated through the inhibitory neurotransmitter Gamma-Amino-Butyric Acid (GABA)." GABA is an amino acid present in the brain of mammals. The central BZ receptors interact with GABA amino acid and change it from active to inactive; "this inhibits the ascending reticular activating system." BZs depress the sensory portion of the brain that controls muscle and motor nerve function. They can blunt emotions and produce what is called "emotional anesthesia," which is the lack of emotional response.$^6$ Benzodiazepines directly affect the diencephalon area of the brain producing sleepiness by relaxing and calming the body or they can slow nervous system transmissions in such a way as to act as an anticonvulsant.$^7$

Despite the similarities among BZs, many drugs in this class are often used for specific treatments, but they are all interchangeable. Often individual drugs are limited by the applications for which their research has been sponsored, but "calling some anti-anxiety drugs and others hypnotics (sleeping pills) has more to do with marketing than with pharmacology."$^8$ For example, Diazepam can be used for short-term management of anxiety disorders, for acute alcohol withdrawal, as an anticonvulsant or a sedative, but it is mainly used as a skeletal muscle relaxant. Alprazolam is primarily used for the symptoms of panic disorders and as an anti-anxiety agent while Temazepam is basically used for short-term management of insomnia.

Although benzodiazepines have many similarities, they do have some differences. The length of their half-life and the tightness of their binding differentiate them. The half-life of a drug is the length of time the drug will remain in the body. Diazepam has a half-life of about 30 hours, Alprazolam’s half-life is about 11-16 hours, and Temazepam’s is about 8-15 hours. Short half-life drugs usually have less protein binding. Diazepam has a long half-life and is 99% protein bound. Alprazolam and Temazepam are short half-life BZs, and they are 90% protein bound.

Drugs that have a shorter half-life usually have inactive metabolites. The binding of BZs to the GABA receptors is most intense in the cerebral cortex. Alprazolam binds
especially tightly increasing its tendency to produce intense sedation, hypnosis, and more severe cognitive deficits, behavioral abnormalities, rebound effects and withdrawal symptoms. Addiction can occur from taking as little as one pill every night for sleep. The tightness of binding to receptors is an indication of how addictive the drug can be therefore, the tighter the drug binds the more addictive it is. “Short half-life BZs lead to rapid drug removal from the blood and brain” and because they don’t remain in the body as long, the receptor sites become uncovered faster which leads to earlier withdrawal symptoms and the need for more of the drug.

Some short half-life drugs have higher risks of causing amnesia, dissociation and other psychiatric symptoms. Halcion (Triazolam) is one of these. It has a short half-life and a greater ability to bind to receptors than some other benzodiazepines. Halcion has a significantly different profile from other BZs due to its greater capacity to bind to protein receptors. When it was introduced, it had the appearances of being a good sedative, but it has proven to be extremely dangerous. It has shown to cause serious cognitive dysfunction, from confusion to delirium, and other behavioral aberrations including psychosis, extreme agitation and sometimes violence toward oneself or others. It is thought to be one of the worst BZs ever produced; Great Britain pulled Halcion off the market because of concerns for its safety. Alprazolam and Temazepam have been preferred because of their shorter half-life; they do not have active metabolites that lead to accumulation, and they have less protein binding than Halcion.

The slight difference in the structures of benzodiazepines changes the way they metabolize. The metabolism of Diazepam is primarily hepatic and it involves demethylation, involving primarily (CYP2C19 and CYP3A4) and 3-hydroxylation (involving primarily CYP3A4); it is extensively metabolized to one major active metabolite desmethyl Diazepam and two minor active metabolites temazepam (3-hydroxy Diazepam) and oxazepam (3-hydroxy-N-diazepam).

![Demethylation of Diazepam to the major product desmethyl Diazepam](image-url)
Alprazolam undergoes oxidative metabolism in the liver. Metabolites are produced but have little or no activity with both active and inactive derivatives being excreted in urine. Temazepam is metabolized by direct conjugation with glucuronic acid to metabolites that are inactive and excreted in urine.

Benzodiazepine use is generally safe, but there are some people who should avoid taking them. There are also drug interactions to be aware of. If a patient has had an allergy to any benzodiazepine, avoid all of them. People who suffer from myasthenia gravis (a disease associated with the wasting of muscles, especially those that enable swallowing) should also avoid BZs. Others who should stay away from this class of compounds include alcoholics, diabetics, and people who have liver or kidney disease, or glaucoma.¹¹

There are drugs one should avoid while taking BZs. These include drugs that can cause sedation such as antidepressants, antihistamines, narcotics, marijuana, tranquilizers, barbiturates, MAO inhibitors and alcohol.¹² Benzodiazepines taken with any of these drugs can cause slow breathing and possibly death. Some drugs decrease the effects of benzodiazepines such as tobacco and cocaine, while other drugs can increase effects, such as erythromycin and contraceptives. Antihypertensives taken with BZs can cause excessively low blood pressure. Diazepam may also increase blood levels of digoxin and cause digoxin toxicity. Toxic effects on the central nervous system can occur when taken with clozapin (an antipsychotic).

The search continues for better, more effective, benzodiazepines that have fewer side effects. One that gave a profile and activity level similar to Diazepam was 7-chloro-5-ethoxy-1-methyl-3H-1,4-benzodiazepin-2(1H)-one.¹³ The pharmacological companies’ marketing strategy aims at people who feel anxious; if a safer BZ can be produced demand will grow. Prescriptions for benzodiazepines are estimated at nearly one hundred million a year in the United States. By targeting people who suffer from anxiety, an unlimited demand will be generated well into the next decade.
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Dovonex:
A Vitamin D3 Derivative

by
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April 23, 1999
Abstract

The natural vitamin D synthesis in man depends on ultraviolet light and vitamin D promotes maturation and differentiation of epidermis cells. (1) Calcipotriene (Dovonex) is a synthetic vitamin D3 analog, the latest therapeutic drug. It is a topical, odorless, non-staining ointment by prescription sold in the United States. Calcipotriene (Dovonex) has been successfully used for treatment of skin cell proliferation (psoriasis).

Dovonex, a topical vitamin D analog, is the latest therapeutic drug used in treating moderate plaque psoriasis. It has been available in the United States, by prescription only, since 1993. Short-term clinical trials have shown Dovonex to be as effective as other leading ointments. Dovonex contains the active ingredient calcipotriene monohydrate, a synthetic Vitamin D3 derivative. In 1986, oral vitamin D analogs were discovered to be effective in the treatment of psoriasis. (2) However, oral vitamin D has limited usefulness due to side effects associated with the large dosages often needed for therapy. Hence, topical calcipotriene provides a safe and effective alternative to oral vitamin D.

Dovonex is available as an ointment, cream, or solution. Dovonex ointment is comprised of calcipotriene 0.005% in a base of dibasic sodium phosphate, edetate disodium, mineral oil, petroleum, propylene glycol, tocophenol, steareth-2, and water. Dovonex scalp solution contains calcipotriene 0.005% and the inactive ingredients: isopropanol, propylene glycol, hydroxypropyl cellulose, sodium citrate, menthol and water. Dovonex cream contains calcipotriene 0.005% in a base of cetearyl alcohol, ceteth-20, diazolidinyl urea, dichlorobenzyl alcohol, dibasic sodium phosphate, edetate disodium, glycerin, mineral oil, petrolatum, and water. In studies of 301 patients who had used Dovonex cream twice daily for eight weeks, 10% had complete clearing and 70% had “marked improvement” in symptoms. The most frequent adverse effects were burning, itching, and irritation, which occurred in 10-15% of patients. (3) Approximately 6% of the topical dose of Calcipotriene is systematically absorbed when applied to psoriatic skin. The distribution of absorbed Calcipotriene and its metabolites are similar to other vitamin D derivatives.
Dovonex topical therapy is aimed at reducing the rate of epidermal proliferation or halting the dermal inflammatory process. Vitamin D promotes maturation and differentiation of the epidermis cells.

Dovonex is indicated for the treatment of plaque psoriasis. However, as with any medication there are many precautions. Do not exceed the prescribed dosage. One may experience elevated blood levels of calcium and/or vitamin D after routinely applying too much Dovonex. Patients using Dovonex should only apply it externally and only to the affected areas, and avoid contact with the face or eyes. Dovonex has been shown to cause local irritation when placed in contact with the face. Burning, itching, dryness, irritation, peeling or redness of skin may occur. For extreme cases swelling and worsening of psoriasis can occur also. Other side effects can include: abdominal or stomach pain, constipation, depression, loss of appetite, loss of weight, muscle weakness, nausea, thirst, tiring easily, and vomiting.

Patients with history of hypersensitivity to any of vitamin D analogs, history of hypercalcemia, or evidence of vitamin D toxicity should avoid its use. To avoid hypercalcemia, Calcipotriene use should not exceed 100 grams per week. Chronic hypercalcemia can lead to generalized vascular calcification, nephrocalcinosis, calcifications of the cornea or other soft tissues. Dovonex should not be used if one is pregnant or nursing. Adverse reactions and overdosage of Dovonex can cause skin irritation around the plaque psoriasis or elevated serum calcium. Elevated serum calcium levels should not exceed 70 mg/dL. (4)

Psoriasis is a non-contagious, incurable, persistent skin disease. There is some evidence of a genetic component. Psoriasis is more likely to occur in people whose family members have it. In the United States, 2 out of every 100 people have psoriasis (four to five million people). Approximately 150,000 new cases each year. (5) Evidence also suggests that it is an autoimmune disorder in which the T cells are activated and do not shut off as they normally should. Psoriasis is one of the most common dermatoses. Although it is rarely life-threatening, psoriasis can cause significant morbidity, social embarrassment, financial cost and disruption in patients lives. (6)
Also the cause of Psoriasis is unknown. As noted before, recent discoveries point out an abnormality in the functioning of the white cells in the blood stream triggering inflammation in the skin. This causes the skin to shed itself too rapidly, every three to four days. The skin becomes inflamed, producing red, thickened areas with silvery scales, most often on the scalp, elbows, knees, and lower back. People often notice new spots 10 to 14 days after the skin is cut, scratched, rubbed, or severely sunburned. Psoriasis can also be activated by infections, such as strep throat, and by certain medicines. Flare-ups sometimes occur in the winter, as a result of dry skin and lack of sunlight. (7)

Psoriasis comes in many forms. In some cases, psoriasis is so mild that people do not know they have it. Each differs in how bad it is, how long it lasts, where it is, and in the shape and pattern of the scales. However treatment is based on a patient’s health, age, lifestyle, and the severity of the psoriasis. Different types of treatments and several visits to the dermatologist may be needed.

Psoriasis most commonly affects the elbows, knees, groin and genitals, arms, legs, scalp, and nails. It will most often appear in the same place on both sides of the body. The most common form begins with little red bumps. Gradually these grow larger and scales form. While the top scales flake off easily and often, scales below the surface stick together. When they are removed, the tender, exposed skin bleeds. These small red areas then grow, sometimes becoming quite large. Patients with psoriasis may notice that there are times when their skin worsens, then improves. Conditions that may cause flare-ups include changes in climate, infections, stress, and dry skin. Vitamin D derivatives have been established to control plaque psoriasis.

Chemically, Dovonex (calcipotriene monohydrate) is (5Z, 7E, 22E, 24S)-24-cyclopropyl-9,10-secochola-5,7,10(19), 22-tetraene-1,3,24-tiol monohydrate. An empirical formula of C_{27}H_{40}O_{3}, with 77.84% C, 10.64% H and 11.52% O.
Calcipotriene is a white crystalline powder with a molecular weight of 416.2 grams.
Calcipotriene is slightly soluble in methanol, ethanol, ethyl acetate, and THF.
Calcipotriene has a melting point of 111-115 C and is also air and light sensitive. Shown below is its structural formula (8):
Calcipotriene is a structural analog of 1-alpha, 25-hydroxy-vitamin D2 (Calcipotriol). It inhibits cell proliferation and stimulates cell differentiation. The absolute configuration of the skeleton is known from the synthesis route starting from vitamin D2. This structure was determined using single-crystal X-ray diffraction methods.

Absolute Configuration of Dovonex
The commercial preparation of calcipotriene gives a well defined reproducible crystal modification. Suitable crystals are isolated by successive recrystallization from water-saturated ethyl acetate giving a monohydrate. The crystal packing in calcipotriene monohydrate is influenced by hydrogen bonds. The water molecule donates its two protons to two O(24)-H groups from molecules related by the symmetry of the axis. In the 25-OH vitamin D3 monohydrate the water plays an equivalent role connecting 25-OH groups. One of the hydroxy groups (O3-H) of the cyclohexane ring is hydrogen bonded to the O(1)-H of a molecule related by translational symmetry along the axis. The O(1)-H group donates a proton to the water molecule. (9)
Biologically, the natural supply of vitamin D depends mainly on exposure to the ultraviolet rays of the sun for conversion of 7-dehydrocholesterol to vitamin D3 in the skin. But when that does not occur, Calcipotriene synthetically converts vitamin D for the body. Calcipotriene metabolism following systemic uptake is rapid and occurs via a similar pathway to the natural hormone. 1,25-dihydroxyvitamin D is considered to be the biologically functioning form of vitamin D.

The major functions of vitamin D are to increase the efficiency of intestinal calcium absorption and to mobilize calcium stores from bone in order to maintain the serum calcium and phosphorus concentrations within the normal physiological range. Calcipotriene (Dovonex) results in the inhibition of cell proliferation and induction of cell differentiation in psoriatic skin.

Referring to figure above, synthesis begins with vitamin D being metabolically activated in the liver and the kidney before it is fully active on its target tissues. Synthesis continues when vitamin D and its metabolites are transported in the blood, bound to specific plasma proteins. The active form of the vitamin D (1,25-dihydroxy vitamin D3) is known to be recycled via the liver and excreted in the bile. Synthesis continues with the vitamin D precursors (7-dehydrocholesterol) which are converted to vitamin D3 in the skin by exposure to ultraviolet light. Calcipotriene binds to vitamin D receptors on epidermal cells and tissue cells. Vitamin D is converted to 25-hydroxyvitamin D and then to 1,25-dihydroxyvitamin D in the kidney. (10)

The major functions of vitamin D are to increase the efficiency of intestinal calcium absorption and to mobilize calcium stores from bone in order to maintain the serum calcium and phosphorus concentrations within the normal physiological range. Calcipotriene (Dovonex) results in the inhibition of cell proliferation and induction of cell differentiation in psoriatic skin. (11)

In humans, our bodies are capable of making vitamin D when exposed to sunlight on the skin. Sunlight acts on 7-dydrocholesterol in the skin, converting it to vitamin D3, (cholecalciferol). In the liver, an enzyme reaction converts vitamin D3 into 25-hydroxycholecalciferol (25-OHD3) which increases its potency by a factor of five. In the kidneys another enzyme reaction converts 25-OHD3 into 1,25-dihydroxycholecalciferol (1,25-(OH)2D3) which further increases its potency by a factor of ten. Calcipotriene is a synthetic analog of vitamin D3. 1,25-dihydroxyvitamin D2 and its analogs have recently been shown to be valuable in treating the skin disease, psoriasis. (12)
Calcipotriene (Dovonex) has been proven to reduce skin plaque formation in psoriasis. It combines its potent effects on cell proliferation and differentiation giving it a non-evasive, comfortable way to combat moderate plaque psoriasis. Research will continue in the direction of establishing vitamin D derivatives and analogues which have retained their regulatory effects on psoriasis.
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Androstenedione: A Drug That Builds or A Drug That Kills

April 23, 1999

By: Teg Butler
Dr. Mancini’s class; Spring, ‘99
Abstract:
Androstenedione is a product that can be sold over the counter. The supplement is used to help build muscles by increasing the amount of testosterone in the body. Evidence supports that the use of this drug is not conclusive to sell due to lack of research, and therefore should not be sold over the counter for this use.

“Our product is pharmaceutical grade, purified Androstenedione, proudly made in the USA and it is the same stuff used by many professional athletes. Andro is quickly becoming one of the most trusted and effective supplements on the market. It is 100% legal and natural with no known side effects. Androstenedione is considered a natural steroid because it is legal and safe but has many of the same positive effects of illegal steroids. For this reason, many people say it is a miracle for athletes and bodybuilders! Want proof? One of the best home run hitters of all time uses it daily! We GUARANTEE our product to be at pure high quality Androstenedione or TRIPLE your money back! One bottle will last you about one month on average. We have many customers who order from us each month over and over again,” says Peak Nutrition, who are the major manufactures of androstenedione.¹

Propaganda like this are posted in all advertisements that sell androstenedione. even though the producer says it is safe to use, are they right? Does androstenedione have side effects? Of course Peak Nutrition is going to offer triple their money back because it does work, but for how much and risk are you willing to put your life in danger of taking this drug? questions like this will be answered along with the history of androstenedione, structure determination, the reaction of androstenedione when introduced in the body, the natural precursor to androstenedione, and the possible side effects (if any) to taking the drug.

Even though androstenedione has been widely publicized in the past few years its synthetic discovery was back in 1935. Charles Kochakian, M.D., who was a expert in steroid hormones detected that the drug had two kinds of effects, androstenedione was androgenic (creates muscles) and had anabolic possessions. This breakthrough in science was ignored for almost thirty years until two doctors, R.B. Greenblat and V.B. Mahesh found that androstenedione had a major rise of testosterone levels in the body. a regular dosage of a hundred grams to a adult male produced over three and a half times the normal level of testosterone in the body with the drug. Androstenedione has this effect for over three hours, reaching its peak at about forty-five minutes to an hour after taken orally. Now, these effects were also detected in women. Other attempts around this time were merely successful to this breakthrough. For instance, androstenediol, a derivative of androstenedione was tested. Only a meager forty-five percent increase of testosterone was present. In the 1970’s, the former East Germany vitalized Androstenedione by liquidating it into a nose inhaler in a “Attempt to boost the performance of Olympic swimmers and other athletes.”² And finally, the revitalization of androstenedione was widely known with the home run chase of Mark McGuire and Sammy Sosa (both playing in Major League Baseball) to reach the unprecedented mark of sixty-one home runs previously set by Roger Maris in the 1961 season.
The Drug is banned in many professional sports. Organizations like the NCAA, the Olympics, and the NFL ban Androstenedione or any derivative of it. However, the drug is not banned in professional baseball. This is even after the 1998 season was over and Mark McGuire crushed his record 71st home run on October 4, 1998 to take the home run title in his last game of the season.

Androst-4-ene-3,17-dione (Androstenedione)

This is the chemical structure of the drug androstenedione. Androstenedione is also called “androsten,” “stene,” and the most popular name that is used to sell over the shelf “andro.” The bolded arrows indicate carbon bonds included in the molecule that go up (out of the paper in 3D terms). The hydrogen included in the bolded arrows, the middle arrow, show a bond with that carbon. The “4” indicates the double bond at the 4 positioned carbon from the top of that particular cyclohexene. The “3” and “17” indicate the oxygen’s at the third and seventeenth carbons in the molecule. The carbon numbers to count where the structure determination goes is by this.

This Structure has a molecular formula of C19H26O2. It has a molecular weight of 286.413, and has a melting point of 173-174 degrees Celsius. In its physical description at room temperature, androstenedione’s appearance is in crystal form.

Most of the different bond angles are pretty much the same when it comes to calculating cyclohexane’s. For the cyclohexane’s in the molecule, the average distances from each carbon is around 1.563 Angstroms (Angstroms denounded by the abbreviation “A” with a circle on top). The second cyclohexane is being introduced in the model below to show a major functionality that is common in many other anabolic steroids. Here are the distances of the carbons in the molecule in that particular cyclohexane:
The Distance exposed in light gray shows that special carbon (the 6th carbon and 7th carbon bond) is a smaller distance by more than a third of the average distance in the molecule, and about a sixth smaller than the average length in the cyclohexane shown. This is common in many other anabolic steroids that are taken like Androstenedione.4

Androstenedione is a adrenal hormone that is produced naturally in males and females from the adrenal glands either from the ovaries or testes. The natural secretion of it is primarily done in the morning between the time before you wake up in bed to about the time you are done getting ready in the morning, basically about a half hour. This is either produced into testosterone or estrogen, whichever the body needs. Androstenedione is also a metabolite (product of metabolism) of DHEA.5 DHEA is a natural producing drug that is commonly found in young growing males. The Structure of it is closely related to Androstenedione. The differences are the ‘bolded arrow’ bond with the hydrogen attached to it is gone, and the double bond with oxygen located at the “3” position is converted to a ‘bolded arrow’ bond along with a hydrogen attached to it. The structure of DHEA looks like this:

Androstenedione is also derived from pine pollen (in its derivative form androstenediol). It is extracted from the pine tree, and then introduced in the lab with acetic acid in cold conditions (35-40 degrees Celsius) along with a little chronic acid. This solution is kept under 45 degree Celsius conditions for about a half hour. Then the solution is mixed with water and removed with ether. From that, the ether solution is washed with sodium carbonate solution. When cooled, the solution yields crystals and then filtered. The finishing product is androstenedione.6
Androstenedione can be sold over the counter because it is an extract from a plant. Due to the Dietary Supplement Health and Education Act of 1994, which categorizes any "natural" supplement (one derived from animal or plant extracts), rather than synthetic chemicals can be classified as a food, not a drug. Therefore, the drug is unregulated. This issue can be largely debated due to the ban of marijuana and cocaine which are also derived from plants. However, the reason androstenedione can be used legally is due to the natural precursor that is produced like briefly discussed earlier naturally in the body, as opposed that marijuana and cocaine are not.

The chemical reaction when introduced in the body goes as such: Androstenedione is the precursor to making testosterone in the body. The drug is taken orally and exerts its anabolic effect as a result of an enzyme conversion reaction in the liver. When you take Androstenedione an enzyme in the liver acts on the molecular structure of Androstenedione, and through this one reaction converts it to testosterone. This conversion only requires one enzyme reaction and appears to convert only to testosterone. This fact is important as there are other substances that convert to testosterone but also convert to estrogen's as well. Blood levels start rising about 15 minutes after oral administration of androstenedione and stay elevated for around three hours. A peak in blood testosterone levels is seen around one to one and a half hours after ingestion. At the same time, natural testosterone that is produced in the body is shut down, and doesn't produce in the body causing men to be infertile. This is where the reaction to where androstenedione is converted into testosterone:

![Chemical Reaction Diagram](image)

Elevated levels of testosterone stimulate muscle building. This is called the "anabolic" effect, and is where anabolic steroids, which are usually injected directly into the muscle receive their name. A 50mg dosage can increase the testosterone level to about 183% the normal amount produced regularly in the body. The results can be as much as a 400% level increase with a 250mg dosage. The dosage amount is suggested in every bottle sold over the counter. the suggested dosage is anywhere between 50mg and 250mg, but there is no regulated amount to where more or less can be taken. Of course, the more that is taken, the higher the amount of effect can be obtained.
Androstenedione does not produce muscles. What it does is produce more energy in the form of testosterone to give more ‘drive’ for the user to work out longer to activate muscle growth the natural way - by earning it. The effect that it can have depends on many factors. How intensely someone trains, how well someone eats, someone’s natural recuperation ability, genetics, how well someone responds to the supplement are the many factors that come into play when one or more people take it.

“There is no scientific evidence to support using androstenedione to improve athletic performance, says Dr. Edmond Burke, Ph.D., an associate professor of biology at the University of Colorado, Colorado Springs. He is also the director of sports science for the U.S. Cycling Team. Burke goes on to say that “Androstenedione has a short half-life, and the shorter the half-life the less time it spends in the body.” This means that a athlete would have to take the drug continuously on the regular basis to keep the testosterone levels like androstenedione produces.

With the following research, there have been mixed responses to the side effects, short and long-term (if any) to taking androstenedione. As noted in the beginning, Peak Nutrition will guarantee that androstenedione is safe and effective or they will triple your money back. Of course, we cant always assume that what we read is the truth. Dr. Burke explains that there was a “Doping” program that was studied in former East Germany. The side effects in female athletes that were taking similar supplements experienced “muscle tightness and cramps, increased body weight, acne, gastrointestinal problems, changes in libido, amenorrhea (abnormal absence or suppression of menstrual discharges), liver damage, and stunted growth in adolescents.”

Dr. Weindruch, M.D., of the University of Wisconsin, and who has a grant proposal in prostate cancer research says that “Epidemiologic data suggest that diet and serum androstenedione levels may influence the progression of latent forms of prostate cancer into more aggressive prostate cancer.” Weindruch concludes that selling androstenedione may be thoughtless due to the hazards corresponding to usage in the long-term.

Natural excessive testosterone studies (with the aid of taking large amounts of androstenedione), may be “further metabolized to dihydrotestosterone (DHT),” Burke says. There is no muscle building effect with DHT, but has proven to show baldness in males but a increase in body and facial hair, lowered HDL cholesterol, and irregular prostate growth. Furthermore, high DHT levels show a disease called gynecomastia. Even though the name may sound like a deadly disease, it is the enlargement of breast tissue in males. This is where males grow breasts from converting testosterone to the estrogen, estradiol.

In females, androstenediones conversion into testosterone, in turn, mutated to many forms of estrogen, may be responsible for increased cancer in grown women by six times. Among 68 women in the study, all showed this long-term effect. The positive side-effects for women who are targeting for some kind of birth control show a
interference of pregnancy to where ovulation is accelerated greatly. Studies are being done as of the moment to have androstenedione manufactured for women that prevents the drug to form estrogen.

From all of this, Dr. Burk’s conclusion is to advise people who are taking androstenedione for a stretched period of time should, “confirm their prostate health with regular PSA blood tests. They should also discuss the therapy with their physician.”

Young males and females who are taking androstenedione also are at risk of side effects listed above, but one more due to the age. Because young children are producing testosterone and estrogen in the body that is needed to grow to a adult, the shutdown of these natural hormones in the body stunts the growth and age process in adolescents.

From these safety concerns, General Nutrition Centers (GNC), a national chain of nutrition-supplement centers ordered its 3,700 stores not to sell the muscle enhancing pills that baseball slugger Mark McGwire uses back in August. In a June 9 memo obtained by the Chicago Tribune, General Nutrition Centers said its own review of scientific literature concluded that "the use of androstenedione without risk of adverse events cannot be demonstrated." Other chains, however, do not share General Nutrition Centers’ concerns. Great Earth Vitamin stores, a chain of 138 franchises in 23 states, sell it over the counter and by mail order. Androstenedione can also be obtainable over the internet. Just by searching on the World Wide Web, over 20 suppliers sell androstenedione for the same, if not, less that is sold in nutrition stores all over the country.

From the person who started the controversy, the Cardinals’ medical team defended McGwire’s use of androstenedione, saying that it is a "natural substance" with "no proven anabolic steroid effect nor significant side effects." Who says they are right? How can they prove it.

Whatever the case is, androstenedione is a popular product that has proven to give results. Results that can be seen physically to the body, and to statistics on paper. If androstenedione helped Mark McGuire win the home run race last year, then the adverse effects of that is going to make every person who wants that 'edge' in sports to use it. The press did their homework to see what this drug is, and what effects it can have. Yet, they are the ones that are not taking the muscle-enhancing pill. It is now time for the people who are taking or want to take androstenedione to do their homework. They will find some startling results if they do.


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From The Amazon to Modern Medical Settings-
From Blowgun to Hypodermic:
Synthetic Curare—Success.

By

Michelle Daugherty
April 23, 1999
Abstract

Succinylcholine, a powerful yet ultra-short acting neuromuscular blocking agent, is used in hospital emergency rooms, in intensive care units, and in operating rooms. Synthetically made, with the idea of curare in mind, and patterned after acetylcholine, succinylcholine binds to the motor endplate at the neuromuscular junction and prevents rapid degradation of both excess acetylcholine (by acetylcholinesterase)* and succinylcholine (by butryrylcholinesterase).† The effect is muscle relaxation or paralysis. These two terms are used synonymously with each other, but in actuality the effect is best described as muscle paralysis. This paper will look at succinylcholine’s: history and relationship to curare and curare-like drugs; structure and similarity to acetylcholine; chemical and physical properties; mode of synthesis and storage; and dangerous side-effects.

For centuries the South American Indians of the Amazon region have used poison on the tips of their hunting arrows. Each tribe has a name for its “poison,” and collectively the poisons are known as “curare.” At one time curare was worth its weight in silver, and because of this, the preparation was shrouded in secrecy. The botanical sources, or “ingredients,” for curare include roughly 30 plants, but the paralytic agent itself is extracted from one of two plant genera which are entirely different from each other. Most widely used are various species within the genus Strychnos, but some species of Chondrodendron also yield curare.† It is worth mentioning that strychnine is not present in the Strychnos genus,‡ as the name seems to indicate, thus eliminating the possibility of strychnine being the poisoning agent.

The process of making curare is a lengthy and arduous task. At completion, the Indians soak the tips of their arrows in the poison. Administration of the poison is by ejection of the lethal arrow from a bamboo blowgun with a single breath of air. The Indians typically hunt birds and monkeys, but a single dose of curare is said to be able to drop an ox within three minutes. Shortly after intramuscular injection of the poison, the animal is rendered paralyzed. Just prior to using the arrow, the poison is activated by licking the arrow tip. The Indians are aware that the poison’s efficacy exists only when in contact with blood and not with saliva or in the digestive system; therefore, they eat the entire animal without hesitation. However, if an open sore is present within the mouth, one would not want to taste the curare.

Reports by Europeans exploring the Amazon region date back to roughly 1548, and further explorations describe the use of the poison by the Indians. Although the story is told that Sir Walter Raleigh brought the poison back to Europe in the late 16th century, a review of Raleigh’s own writings does not support this tale.

* Acetylcholinesterase is an enzyme that hydrolyzes only choline-ester linkages.
† Butryrylcholinesterase is also known as pseudocholinesterase. It hydrolyzes other ester linkages besides choline-esters.
The pursuit of the Indians’ “secret poison” continued for three centuries. In May of 1800, Alexander von Humboldt and Aime Bonpland were the first scientifically trained people to witness the entire ceremony of curare preparation. In 1812, Charles Waterton went to Guyana with the intent of bringing back the most potent of all curare. He believed that curare had a place in medicine and he was correct. As Waterton predicted, in the late 19th and early 20th centuries, curare attracted the attention of physicians as a possible means of controlling tetanus. Curare was not successful in treating the muscle contractions of tetanus, but it did show some efficacy for treating the convulsions associated with the disease.

In 1935, researchers isolated the pure crystalline alkaloid, \( d \)-tubocurarine chloride from the species of plant known as \( Chondrodendron tomentosum \). They believed the paralytic effect was due to an alkaloid, which incorporated quaternary ammonium groups. After the discovery of the positively charged nitrogen groups, the foundation for creating synthetically made paralytic agents was ready. In 1940, Squibb developed the first standard product ready for medical research. The drug’s generic name is (\( + \))-Tubocurarine Chloride, and the brand name is Intocostrin®. Many products with the bis-quaternary ammonium moiety were made.

Researchers discovered that fragments of hydrolyzed curare-like products contained 10 atoms separating the 2 quaternary ammonium groups. Maximum muscle relaxation occurred when this type of fragment was present, and especially when the distance between the 2 nitrogen cations approached 1.2 nm. The tubocurarine structure seen here has the 10 atom separation identified.

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{CH}_3 & \quad \text{N}\,^1 & \quad \text{H} & \quad \text{OCH}_3 \\
\text{H}_3\text{CO} & \quad \text{OH} & \quad & \quad & \\
\end{align*}
\]

(\( + \))-Tubocurarine

*Alkaloids are compounds mostly of plant origin that even in very small amounts produce strong physiological effects on the body.
With this idea in mind, and knowing the structure of acetylcholine, succinylcholine was soon synthesized. As will be seen, succinylcholine's structure fits the "10 atom separation" description. Medicinal chemists refer to this "atom separation" as "structure-activity relationships" (SARs). These types of relationships are important when identifying and comparing drug efficacy.\(^4\)

Tubocurarine was once used to a great extent in operating rooms around the world. The paralytic properties showed promise as anesthesiologists realized that when muscular relaxation agents were used in conjunction with general anesthetics, lower doses of medication could be used in the patient. Although tubocurarine is not used as widely as it once was, it still holds historical importance.\(^3,5\)

Numerous neuromuscular blocking agents currently exist. Some botanical products include d-tubocurarine and alcuronium. Lab-created neuromuscular blocking agents have successfully replaced the original curare derivatives,\(^*\) with fewer side effects and greater potency. Neuromuscular blocking agents are classified by their by mode of action and categorized by their time of onset, as well as their peak of effectiveness and its duration, which can range from as little as 10 minutes to as long as 2 hours.\(^†\) Depending on the medical procedure, the condition of the patient, and length of effectiveness desired, the physician determines which neuromuscular blocking agent to use.

<table>
<thead>
<tr>
<th>Blocking Agent</th>
<th>Route</th>
<th>Onset</th>
<th>Peak</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubocurarine</td>
<td>Intravenous (IV)</td>
<td>1 min</td>
<td>2-5 min</td>
<td>20-40 min</td>
</tr>
<tr>
<td></td>
<td>Intramuscular (IM)</td>
<td>It is not administered (IM) in medical settings</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Succinylcholine</td>
<td>Intravenous (IV)</td>
<td>30-60 sec</td>
<td>1-2 min</td>
<td>4-10 min</td>
</tr>
<tr>
<td></td>
<td>Intramuscular (IM)</td>
<td>2-3 min</td>
<td>Unknown</td>
<td>10-30 min</td>
</tr>
</tbody>
</table>

\(^*\) Lab-created neuromuscular blocking agents include: vecuronium, rocuronium, pipecuronium, atracurium, metocurine, and mivacurium. All of these drugs are competitive or nondepolarizing agents.

\(^†\) Special circumstances exist where the duration of succinylcholine extends beyond 2 hours, even though it should last a maximum of 10 minutes. Later this phenomenon will be discussed.
With the duration of effectiveness in mind, it is important to reiterate that succinylcholine is an ultra-short acting blocking agent. For this reason, it is very popular in hospital emergency rooms when endotracheal intubation is necessary. The route of administration is almost always intravenous (IV), but it can be given intramuscularly (IM) if necessary.

When succinylcholine is administered it causes the cells to deplete all their acetylcholine, which “over-loads” the motor endplate. The dose of succinylcholine joins the acetylcholine at the neuromuscular junction on the endplate. The phenomenon results in a “mess” much like a truck spilling a load of gravel on the highway and stopping all traffic. Once clean-up crews remove the gravel, traffic can resume. In this case, acetylcholinesterase and butyrylcholinesterase are the “clean-up crew.” (Seen here is a diagram of the motor endplate.)

Many names exist for succinylcholine. Some generic names include: suxamethonium chloride; choline chloride succinate; succincurarium chloride; and 2,2’-sucinylidioxybis(ethyltrimethylammonium) dichloride dihydrate. Brand names include: Quelicin® by Abbott, Scoline® by Duncan Flockhart, Sucosstin® by Squibb, and Anectine® by Glaxo Wellcome. While all of these names are for succinylcholine chloride, medical personnel simply refer to it as “succs.”

Succinylcholine is a depolarizing neuromuscular blocking agent. Unlike most of the nondepolarizing agents, which have large and rigid chemical structures. Succs has a structure that is small and flexible, and because of the its single bonds, it allows...
for free-rotation. Succinylcholine is patterned after acetylcholine, which will be demonstrated by the two structures below. In fact, the compound looks like two acetylcholine molecules attached at the alpha carbons. (Take a moment to compare the structures and refer back to the structure of tubocurarine.)

\[
\begin{align*}
&\text{O} \\
&(\text{CH}_3)_3\text{N}^+\text{--CH}_2\text{CH}_2\text{--O--C--CH}_3 \\
&\text{Acetylcholine}
\end{align*}
\]

\[
\begin{align*}
&\text{O} \\
&3(\text{H}_3\text{C})\text{N}^+\text{--CH}_2\text{CH}_2\text{--OCCH}_2\text{CH}_2\text{CO--CH}_2\text{CH}_2\text{--}^+\text{N(CH}_3)_3 \\
&Succinylcholine
\end{align*}
\]

Also notice that the molecules do not contain any chiral carbons; therefore, stereoisomers do not exist. The quaternary ammonium groups at both ends carry a positive charge; therefore, the overall charge of succinylcholine is \(^{+2}\). The positive 2 charges of succinylcholine are ionically bonded to 2 negatively charged halide ions. Although mostly made with chlorine, succinylcholine can also be synthesized with bromine and iodine.

One type of synthesis involves the use of 1 mole of a diacid chloride reacting with 2 moles of choline. This yields 1 mole of succinylcholine.

\[
\begin{align*}
\text{Cl--CCH}_2\text{CH}_2\text{C--Cl } + \ 2 \ (\text{CH}_3)_2\text{N}^+\text{CH}_2\text{CH}_2 & \rightarrow \\
3(\text{H}_3\text{C})\text{N}^+\text{--CH}_2\text{CH}_2\text{--OCCH}_2\text{CH}_2\text{CO--CH}_2\text{CH}_2\text{--}^+\text{N(CH}_3)_3
\end{align*}
\]

This reaction proceeds readily at room temperature. \(^8\) Conflicting information was given about the use of an autoclave for sterilization. Contamination, by heavy metal ions from the autoclave, was a problem and ions were difficult to remove. \(^9\)
Techniques must be different because an autoclave was utilized and contamination was not addressed as a problem.\textsuperscript{10}

Succinylcholine chloride is a white, odorless, slightly bitter powder, which is highly soluble in water and moderately soluble in very small alcohols. In solution, the pH is about 4, and it is sensitive to alkaline solutions. The percent composition is: C 46.54\%, H 8.37\%, Cl 19.62\%, N 7.75\%, and O 17.71\%, with a molecular formula of $\text{C}_{14}\text{H}_{30}\text{Cl}_2\text{N}_2\text{O}_4$. The total weight of succinylcholine chloride is 361.3 grams per mole. According to the literature, succinylcholine has a melting point of 160-164° C. The broad temperature range is indicative of salts; therefore, impurities are probably not much of a factor.\textsuperscript{7,11}

As seen by the structure, it is evident what the expected IR and NMR would show. "A neat sample on a KBr disc produces the following IR: 1735 cm\textsuperscript{-1} is a carbonyl peak (C=O), 1425 cm\textsuperscript{-1} represents an amine peak (\textsuperscript{14}N-\textsuperscript{1}CH\textsubscript{3}), and 1310, 1150 cm\textsuperscript{-1} represent the ether peaks (C-O-C). The NMR—run at room temperature, in $\text{D}_2\text{O}$ with a DSS internal standard—yielded the following information: a singlet at 2.71 (4H), a singlet at 3.22 (18H), a triplet at 3.65 (4H), a triplet at 4.52 (4H) and a singlet at 4.61 (1H, HDO)."\textsuperscript{8}

Researchers conducted a study to determine the stability of succinylcholine in solution at various temperatures and light conditions. For long term storage, up to 2 years, succs must be refrigerated and left in a light-protected environment. The question became, "How long can succs be kept at room temperature before effectiveness is lost?" Also, "What length of time can succs be kept under lighting before decomposition occurred?"

In hospital settings like the operating room and intensive care unit, storing in a refrigerator and in the dark is not a problem. The answers to the researchers’ questions are vitally important to the staff in hospital emergency departments. Storing in a location away from the medical emergency is not practical because time is crucial.
As already stated, maximum storage is 2 years in a dark refrigerator. However, the variables of light and temperature greatly influenced the storage times. A 10% solution of succs in dextrose and normal saline, stored between 20-26 °C and in the dark, kept 90% of its effectiveness for 5 months. The same solution, stored at 70 °C, had 90% effectiveness after 1 day.\textsuperscript{10,12}

In the emergency room, succs is used frequently to fascilitate endotracheal intubation. Succinylicholine causes the cells at the neuromuscular junction to release all their acetylcholine, thus over-loading the motor endplate. Succs also goes to the endplate with the acetylcholine molecules. After 30-60 seconds, muscle fasciculations occur and paralysis soon follows.\textsuperscript{5,7,11,13}

Butryrlcholinesterase, the enzyme responsible for degrading succinylcholine by cleaving the ester linkages, catalyzes a reaction of 1 mole succinylcholine with 2 moles water. This yields 1 mole succinic acid (a dicarboxylic acid) and 2 moles of choline (a positively charged amino compound). The fast step of the reaction is the hydrolysis of one choline-ester bond with a resulting inactive monoester intermediate.\textsuperscript{10,14} This intermediate is short-lived, and soon the other choline-ester bond is broken, resulting in another choline and 1 succinic acid.

\[
\begin{align*}
\text{C}_{14}\text{H}_{30}\text{N}_2\text{O}_4 & + 2 \text{H}_2\text{O} \rightarrow 2 \text{C}_5\text{H}_{14}\text{NOH}^+ + \text{C}_4\text{H}_6\text{O}_4 \\
\text{Succinylcholine} & \quad \text{Water} & \quad \text{Choline} & \quad \text{Succinic Acid}
\end{align*}
\]

This reaction is so efficient that only some 10% of succs is excreted in the urine. Although no information was located to support this hypothesis, it is possible that the choline molecules enter reactions to become acetylcholine and other needed biochemicals.

As important and powerful as succinylcholine is, it is not without some serious side effects. Many adverse biological reactions have occurred as a result of using succinylcholine, such reactions include: hyperkalemia, the excessive potassium release from the cells; heart rate, either increased or decreased; fasciculations, muscle twitches; myalgia, muscle pain from the fasciculations; and increased intraocular, within the eye, and intragastric, within the stomach, pressures. Hyperkalemia, because of the potential for cardiac arrest, is considered to be one of the most severe reactions.\textsuperscript{7,13-15}

As already discussed, succinylcholine is hydrolyzed by butyrylcholinesterase. Prolonged paralysis occurs in individuals who are homozygous recessive for an atypical gene that prohibits production of butyrylcholinesterase. In patients with a complete lack or diminished quantity of this enzyme, a single dose of succs can render a person paralyzed for hours.\textsuperscript{7,16} Providing that mechanical ventilation is continued, the patient will eventually emerge from the effect of succs without further
complications; however, without airway maintenance and administration of oxygen, the patient can die. Currently, no medications exist that can reverse the effect; in homozygous recessive patients, time is the only counter-measure for succinylcholine usage.

Rare, life-threatening side effects do occur, but negative repercussions possibly can be averted providing that medical staff is alert to the first indications of trouble. For example, Duchenne's Muscular Dystrophy (DMD) is an X-linked recessive trait. Males inherit their X-chromosome from mom and their Y-chromosome from dad; consequently, males express DMD since they have only one X-chromosome. The disease is typically not evident until the boy is a couple of years old. To the physician, this poses a problem because no evidence of DMD is observable. If a dose of succ is administered, the potential for cardiac arrest from hyperkalemia is present. Medical personnel should be ready to treat the patient for hyperkalemia* and cardiac arrest. 17,18

While succinylcholine is not perfect, currently alternative medications are not abundant. In fact, it is only one of a very few ultra-short acting neuromuscular blocking agents available. Anesthesiologists have long recognized the need for a noncompetitive neuromuscular blocking agent that has the rapid onset, peak, and depletion time of succ. For now, succinylcholine is the only real option available to physicians in the various hospital settings. Until a drug is introduced that can fully replace succinylcholine, risk-benefit assessment will dictate how and to whom it can and will be administered.

* Sodium bicarbonate is given as one measure to treat hyperkalemia.
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SECRETIN

Just another hormone
or
THE CURE?

by
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April 23, 1999
Abstract

Secretin is a heptacosapeptide released in the upper small intestine. Synthesized using fragment condensation, secretin has a helical structure and a conformation that is locally well defined in Me₂SO. This hormone is FDA approved for applications in gastrointestinal diagnostic procedures. Recent discoveries indicate a connection between neurotransmitters and this hormone within the brain. Therefore the implications for the use of therapeutic infusions of secretin in disorders with neurological elements such as Autism Spectrum Disorder should be explored.

It all started with a dog and a bell. The early work of Pavlov described how the motility and secretion of the gastrointestinal tract was regulated by the nervous system. However, it was noted early on in his studies that despite cephalic stimulation, little substance was secreted from the pancreas without the presence of an acid chyme.

It was this observation that led Bayliss and Starling to the discovery of secretin in 1902. They were actually looking for a link between the peripheral nervous system and intestinal function but ended up discovering the first hormone. This discovery occurred experimentally. While observing the gastrointestinal tract of an anesthetized dog, it was seen that when an acid chyme was introduced into the system, secretions began to occur from the pancreas at a rapid rate. The hormone was thus named secretin due to its ability to stimulate the secretion of pancreatic juice. This discovery eventually led to the establishment of the field of endocrinology.

After the discovery of secretin, sixty years would pass before Jorpes and Mutt in 1961, would isolate it in a pure form through extraction from a pig, whereby they later would identify the amino acid sequence. Five years later in 1966, Bodansky, et al., synthesized the hormone. Secretin has been discovered in nearly all vertebrates, as well as non-vertebrate species, such as the octopus. Secretin has been isolated from four different vertebrates but only those of human and porcine will be covered, as these are the only sequences that have been extensively studied. Biological implications will be addressed as they relate to humans.

In comparing human and porcine secretin, the immediate difference can be seen in the structures of the two. Human secretin has a residue structure of:


Porcine secretin differs from the human molecule in two positions, with regard to Asp for Glu at 15 and Ser for Gly at 16. Currently porcine secretin is available from Ferring Laboratories in a highly purified naturally occurring form, extracted from the duodenum of pigs. Synthesized for research studies in both human and porcine form, the porcine form has a molecular weight of 3055.5 daltons and an empirical formula of C₁₃₀H₂₂₀N₄₄O₄₁.³
Several different methods of synthesis have been attempted with secretin. Due to instability factors the most commonly employed method today is the procedure developed by R. B. Merrifield of Rockefeller University. This method, utilizing a polystyrene resin containing a \(-\text{CH}_2\text{Cl}\) group, earned him the Nobel Prize in 1984. This type of synthesis led to the discovery of additional causes of instability.

Initially it was believed that the instability of secretin was due only to the succinimide formation and the $\alpha \rightarrow \beta$ transpeptidation at Asp3 residue. However, upon further investigation, it became apparent that there are other structural changes causing this phenomenon. Through the alternative synthesis of secretin, it was found that His1 is crucial to full biological activity. Possessing an unusual release pattern in an aqueous solution, histidine was an essential player in the instability of the molecule.

The other factor in the instability equation was determined to be the Asp-X bond, where X equals Gly or Ser. The bond, which occurs twice in the human molecule, and three times in the porcine is very susceptible to hydrolysis. These problems must be a consideration when synthesis is undertaken. The diagrams that follow depict a synthesis that entailed the condensation of four peptide fragments.

\[
\begin{align*}
4 \text{Z(OMe)-His-Ser-Asp-Gly-Thr-Phe-NHNH}_2 \\
3 \text{Z(OMe)-Thr-Ser-Glu-Leu- NHNH}_2 \\
2 \text{Z(OMe)-Ser-Arg-Leu- NHNH}_2 \\
1 \text{Z(OMe)-Arg-Asp-Ser-Ala-ArgLeu-Gln-Mts} \\
\text{Arg-Leu-Leu-Gln-Gly-Leu-Val-NH}_2 \\
\end{align*}
\]

**Synthetic Route to Porcine Secretin**
For fragment [1], a protecting group was employed to prevent base-catalyzed succinimide formation of the Asp residue. The side chain protecting group was removed at the stage of each fragment synthesis. Arg(Mts) was used as a protecting group because it is known to cleave smoothly with TFMSA-thioanisole in TFA. The active ester procedure was used for the introduction of the corresponding amino acids. An azide method was initiated for the dipeptide hydrazides. Due to the concern of succinimide formation, after the addition of the Boc-group, the Bu-group as well as the Boc-group was removed using TFA before condensation of the additional fragments.

Fragment [2] was prepared by azide condensation. Fragment [3] was synthesized by utilizing the NP method, hydrogenolysis and the azide method. Fragment [4] is the N-terminal fragment and was prepared in a stepwise manner as well as by preparing a protected hexapeptidyl ester, which was converted to the corresponding hydrazide.

After the preparation of the fragments, condensation followed using the azide method to produce the heptacosapeptide amide chain of porcine secretin. Each condensation is performed in DMF or DMF/DMSO 4:7 and the products purified using silica gel filtration. Subsequently, purity of every intermediate was assessed using thin-layer chromatography. The protected molecule was then treated with TFMSA-thianisole/TFA in the presence of m-cresol to remove the protecting groups. This deprotecting procedure is performed three times to ensure that the process is complete.

Purification is then undertaken in several steps, which include gel filtration, ion exchange chromatography and reverse phase-high performance liquid chromatography. Purity was confirmed as stated previously. Synthetic porcine secretin was produced that was homogenous and pure, with a yield of twenty-three percent. The synthetic was found to possess full biological activity when the dry powder was dissolved in 7.5ml of sodium chloride injection USP and injected into a rat.

Reconstitution of secretin has been a concern for pharmacological companies. To date, secretin is stored as a lyophilized powder at $-20^\circ$C as it appears to lose biological activity if stored in an aqueous solution. The conformation of secretin was studied in two different solvents to determine if its structure was solution dependent. Secretin belonging to the glucagon family, shares many characteristics with it as they are sequentially related hormones.

Since secretin is now known to have broad biological impact, it was prudent to discover any differences that might distinguish it from other hormones. In reduced water activity, many hormones prefer an ordered structure. The structure of secretin was reasonably expected to have a $\alpha$-helical structure in trifluoroethanol/water. This would be consistent with the other members of the glucagon family. By analyzing secretin in dimethyl sulfoxide solution, comparisons could be made in regards to structure due to solvent effect. NMR technology was essential in drawing these comparisons.
Fig. 5. Homogeneous conformation over all structures of secretin of the Loop region of residues 2–7 from molecular dynamics. The hydrogen bonds of the NH protons of D3, G4, and T5 to the T5-OH oxygen are indicated with dotted lines.

Fig. 6. Homogeneous conformation over all structures of secretin of the region of residues 12–18 from molecular dynamics. Only the side chains of the R18 and D15 residues, which are forming a salt bridge, are shown. The hydrogen bonds of the NH protons of R18 and A17 to the R14 carbonyl oxygen are indicated with dotted lines.

Fig. 4. Superimposition of secretin backbone structures after 3-ns molecular dynamics. (a) Best fit of the Cx atoms of residues 13–21; (b) best fit of residues 2–26 and (c) best fit of residues 3–7.

Conformation Structures of Secretin
H-NMR measurements were done on the molecule, using a synthetic sample. Instead of the reported α-helical structure found in trifluoroethanol/water, none was found in dimethyl sulfoxide solution. It was observed that the “carboxy-terminal moiety of secretin is characterized by a sequence of consecutive turns, resulting in an alternate helical-type structure. The Arg14 carbonyl group is involved in hydrogen bonds to Ala17 and Arg18, forming a kind of β/γ-turn equilibrium. Moving forward in the chain, turns are formed with the following H-bond pairs indicating an irregular helix.”

\[
\begin{align*}
\text{Leu19} & : \text{C=O} & \text{HN Leu22} \\
\text{Arg21} & : \text{C=O} & \text{HN Gly25} \\
\text{Gln20} & : \text{C=O} & \text{HN Leu23} \\
\text{Leu22} & : \text{C=O} & \text{HN Leu26}
\end{align*}
\]

As a result of two correlated two dimensional NMR techniques, the following conclusions about the structure of secretin in dimethyl sulfoxide solution was asserted:

1. The hydrogen bond between Arg14 Ala17 indicates a β-turn conformation.
2. Two well-defined regions between residues 2-8 and 14-20 show interesting conformational features concerning side-chains which are known to be important for biological activity.
3. The Asp15 side-chain carboxyl group forms a salt bridge to the Arg18 guanidino function.
4. The N-terminal region, including the loop between residues 2-7 is well studied and of major importance for the biological activity of secretin. This region reveals a very distinct side-chain orientation.

In this solution, secretin has a conformation that is locally well defined. However, it appears that several different global conformations are possible. The structure of secretin in dimethyl sulfoxide solution also clearly shows structures for side-chain orientation. This phenomenon was not observed in trifluoroethanol/water. The structural differences noted correlate appropriately to amino acid residue locales that are known for their biological significance. The activity of secretin seems to rely on these few fragile areas previously discussed. Considering its areas of fragility, what impact does the structure of secretin have in the body? What role does it play?

The mechanisms of secretin are not yet known. Yet, through careful observation, the process of action seems to be uncontested. Located in the S-cells of the mucosa of the upper intestine, secretin resides in an inactive state called prosecretin. Within the duodenum and jejunum, the prosecretin waits for a catalyst - the acid chyme, to act. Composed primarily of hydrochloric acid and fatty acids, acid chyme enters the duodenum from the stomach. When the chyme possesses a pH of 4.5-5.0, the prosecretin is released and activated. Activated secretin, absorbed into the bloodstream, causes the pancreas to secrete its juice. Pancreatic juice is high in concentrations of bicarbonate ions and low in chloride ions.

This process is especially significant for two reasons. First, the release of secretin is triggered only by acid chyme within the aforementioned pH range. When the pH level
drops and the area becomes more acidic, pancreatic juice secretions become markedly abundant. This precipitates the following reaction to occur in the duodenum.

\[ \text{HCl} + \text{NaHCO}_3 \rightarrow \text{NaCl} + \text{H}_2\text{CO}_3 \]

Immediately following, the conversion of carbonic acid, carbon dioxide is expired out of the body. The duodenum is then left with a neutral solution of sodium chloride. Its contents effectively neutralized, the pecticity of the gastric juices is blocked. This process thus prevents the formation of duodenal ulcers.

\[ \text{H}_2\text{CO}_3 \rightarrow \text{CO}_2 + \text{H}_2\text{O} \]

Secondly, with the bicarbonate secretion of the pancreas, an acceptable pH level is obtained somewhere between neutral and a pH of 8.0. This environment allows for the function of pancreatic enzymes, which are acid sensitive, to have optimal function.

In addition to this mechanism, for which secretin is most widely known; secretin has been found to have roles in the function of the stomach. Secretin inhibits gastric gland secretion and motility during this phase. Secretin potentiates the action of cholecystokinin and increases bile output from the liver. In addition, secretin influences the release of insulin and the growth hormone releasing factor.

Clinically, secretin has been approved by the Food and Drug Administration as an injectable hormone. The approved-label uses consist of three diagnostic tests, for Zollinger-Ellison Syndrome and Pancreatic Exocrine Disease. In addition, within label guidelines is the so-called "Secretin Test," a process by which desquamated pancreatic cells are obtained for cytopathological examination. These diagnostic procedures occur during the process of an endoscopy, a surgical process used to determine disorders of the gastrointestinal system. Until 1996, the approved uses for secretin appeared to be the only valid ones. Then there was Parker Beck.

Parker Beck was a three and a half-year-old boy, clinically diagnosed with autism. In April of 1996 during the course of an endoscopy, the secretin test was administered. The results of Parker’s endoscopic diagnostic evaluation were normal. What happened to him afterward was not. Before the endoscopy, Parker was non-verbal, had difficulty sleeping, had no eye contact, was inattentive and had chronic diarrhea (i.e. the endoscopy). Within three weeks Parker was speaking in full sentences, slept through the night, maintained eye contact 75% of the time and could follow 1-2 step directions. Not only did his diarrhea disappear but also the child who had been in diapers before was toilet trained completely within three weeks.

There was nothing to blame for his remarkable improvements but secretin. No one could believe it, but a few did. Physicians with autistic children, were the first to listen. The million-dollar question for doctors of the 450,000 American children with autism became “Why and how does secretin affect these changes?” Parents asked, “Is it ‘The Cure’?”
Ironically, both the disorder of autism and secretin are enigmas. Both child and hormone are working in a world which no one else fully understands. Though startling new discoveries have been made in regard to secretin, the chemical mechanisms, the *whys* and *hows*, have not yet been determined.

The University of Maryland, where Parker Beck’s endoscopy was performed, later published this account, postulating a hypothesis. The abstract follows:

“We report three children with autistic spectrum disorder who underwent upper gastrointestinal administration of secretin to stimulate pancreaticobiliary secretion. All three had increased pancreaticobiliary secretory response when compared with nonautistic patients (7.5 to 10ml/min versus 1 to 2ml/min). Within five weeks of the secretin infusion, a significant amelioration of the children’s gastrointestinal symptoms was observed, as was a dramatic improvement in their behavior, manifested by improved eye contact, alertness, and expansion of expressive language. These clinical observations suggest an association between the gastrointestinal and brain function in patients with autistic behavior.”

For the doctors, research scientists, pharmacologists and endocrinologists, the answers have not come easily. No one knows why secretin has this impact on some but not all autistic children. Why does it affect them at all? One of the largest obstacles in the path is that the etiology of autism is *unknown*. Autism, a seemingly neurological disorder is a severe impairment affecting many if not all aspects of life.

Afflicting children between the ages of 18-36 months of age, autism is a life-long disability and there is no known cure. This creates a situation where parents and clinicians are desperate and willing to do anything necessary to save these children, including raising the money for the necessary research or for paying corner-market physicians top dollar to infuse their children. Obviously, research seems the safest and surest way to proceed before experimenting with the drug.

In regards to autism, links to secretin and the brain are of particular interest. A study done in 1998 from Harvard Medical School, may have established a gut-brain link. The article abstract follows:

Secretin and vasoactive intestinal peptide (VIP) are known to stimulate tyrosine hydroxylase (TH) activity acutely in the rat superior cervical ganglion (SCG). Because TH-containing neurons in the SCG innervate iris, submaxillary gland, and pineal gland, we examined the effects of secretin and VIP in these 3 autonomic end organs in vitro. Both peptides stimulated TH activity in each tissue. These stimulations resembled those in the SCG in that (1) secretin displayed a higher potency than VIP in all 3 end organs, (2) the peptide effects were unchanged when calcium was excluded from the incubation medium, and (3) they were mimicked by activators of the cyclic adenosine monophosphate (cAMP) pathway. These findings indicate that secretin and VIP can regulate transmitter metabolism in both the cell bodies and axon terminals of neurons originating in the SCG. Furthermore, the data raise the possibility that catecholamine synthesis is
sympathetic nerve terminals is modulated by peptides released by other nearby nerve endings.\textsuperscript{12}

\textbf{Tyr} \rightarrow \textbf{L-Dopa} \rightarrow \textbf{Dopamine} \rightarrow \textbf{Norepinephrine} \rightarrow \textbf{Epinephrine}\textsuperscript{14}

\[\begin{array}{c}
\text{CH}_2
H_2N- \text{C-COOH} \\
\text{OH} \\
\end{array} \rightarrow \begin{array}{c}
\text{HO} \\
\text{HO} \\
\text{CH}_2\text{CH-}\text{NH}_2 \\
\text{COOH}
\end{array} \rightarrow \begin{array}{c}
\text{HO} \\
\text{HO} \\
\text{OH} \\
\text{CHCH}_2\text{-NH}_2
\end{array}

\text{L-Dopa}

\text{Tyrosine}

\[\begin{array}{c}
\text{HO} \\
\text{HO} \\
\text{CHCH}_2\text{-NH}_2
\end{array} \rightarrow \text{Dopamine} + \text{CO}_2 \rightarrow \begin{array}{c}
\text{HO} \\
\text{HO} \\
\text{OH} \\
\text{CHCH}_2\text{-NH}_2
\end{array}

\text{Norepinephrine}

\[\begin{array}{c}
\text{HO} \\
\text{HO} \\
\text{CHCH}_2\text{-NH}
\end{array}

\text{Epinephrine}

Thus, the action of tyrosine hydroxylase is stimulated by secretin, causing the production of dopamine and norepinephrine, both neurotransmitters, from the brain. Biological synthesis in the body itself accounts for the changes between these molecules.
These changes include the addition of the hydroxy group into the 3' position of tyrosine to form Dopa followed by the loss of a carboxyl group leading to dopamine. Loss of the hydroxy group from dopamine gives norepinephrine, and the addition of a methyl group to norepinephrine leads to epinephrine. The mechanism of secretin to stimulate tyrosine hydroxylase is uncertain, and therefore it is not shown.

Secretin and its receptors have been found in the brain, indicating a possible role in the central nervous system via neurotransmitters and/or neuroregulators. Many authorities claim that secretin classifies as a neurotransmitter in its own right. Secretin stimulates the accumulation of cAMP in brain cells consisting of mainly glioblasts suggesting a regulatory on concentrations of cAMP in the brain. The increased levels of dopamine and the increased function of cAMP might account for the drastic changes in awareness and attentiveness in these children.

The million-dollar question for clinicians may be simple. The reaction of secretin in the body increases dopamine levels from tyrosine. Possibly secretin increases levels of serotonin in the brain, a phenomenon observed but not well documented. This would facilitate increased awareness and attentiveness. Could it be so simple as children not getting the required essential amino acids in their diets? Six essential amino acids, two of them particular to children, account for fifteen places in the secretin molecule. Autistic children are generally inclined to have very selective eating habits. The answer to these questions will only come from billions of dollars in research, education and investigation.

For parents, one would hope it is this simple. For these individuals, the hope that secretin be the cure, so desperately sought, would literally be a lifesaver. Yet, not to be proclaimed a cure out of desperation but by utilizing thorough scientific investigation.

Composing a completely accurate depiction of secretin is difficult. Though it was discovered almost a 100 years ago, little is really known about its mechanisms in the body. It is only with the aid of sophisticated technology that we have made any progress with it at all. Once secretin is released in the body, what does it do? How does it do it? Only part of these questions can be answered. Here one must ask the questions: What do we know? What do we think? What can we prove? Then maybe we can answer definitively, is it 'The Cure'?
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LECTINS: The Physiological Effects of Phytoagglutinins Within the Human Body

April 23, 1999

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Abstract: Lectins are glycoproteins that have many distinguishing properties yielding broad applications within the biomedical field. Most notable is the selectivity of certain lectins that allows them to agglutinate specific human erythrocytes. As a result of this property, lectins have been used to type blood, but the physiological effects within the body after ingesting lectins is also being studied. These ramifications have both positive and negative results.

Definition
Lectins are defined as non-catalytic, carbohydrate-binding (glyco)proteins of non-immune origin, capable of specifically recognizing and reversibly binding to carbohydrates without altering their covalent structure. They are also distinguished by their ability to precipitate polysaccharides, glycoproteins and glycolipids. The word lectin comes from the Latin *lego* or *legere* which means to choose or pick out. Lectins are also known as agglutinins, phytohemagglutinins, phytogalactomannin and proteins. Although they were first discovered and most abundant in plants, lectins are also found in almost all other living organisms including mammals, bacteria, viruses, invertebrates, marine organisms and fungi.

Molecular Characteristics
Lectins constitute a heterogeneous group of proteins with a wide range of molecular properties including molecular weight, molecular structure, amino acid composition and other distinguishing characteristics. There are no structural features common to lectins except they are all proteins and consist of subunits or protomers. Most lectins contain covalently bonded sugars, therefore can be classified as glycoproteins. Their molecular weights range from 26,000 gm/mole for wheat germ agglutinin to 400,000 gm/mole for the horseshoe crab lectin. They also can undergo complex association-disassociation reactions which result in multiple forms of the same lectin that differ in molecular weight. Lectins usually consist of 2, 4 or 6 subunits, although some lectins have as many as 20 subunits. Subunits are usually identical, having the same sugar specificity, and each of them containing a single polypeptide chain (sometimes two) with a single sugar binding site. Each lectin molecule has at least 2 carbohydrate binding sites to allow crosslinking between cells. Lectins require the presence of Mg\(^{2+}\) and Ca\(^{2+}\) to bind and agglutinate cells.

The following descriptions serve to demonstrate the varying molecular characteristics and chemical and physical properties of lectins:

*Concanavalin A* (jack bean) is a lectin with a globular protein subunit of overall dimensions 42 X 40 X 39 Å. The crystallographic asymmetric unit has a molecular weight of 25,500 gm/mole and is composed of 238 amino acids; four identical subunits interact to form a pseudotetrahedral molecule. The extended polypeptide chain is arranged in two anti-parallel sheets or B structures (see Figure 1). Concanavalin A has no sugar residues and is therefore a protein, not a glycoprotein.
*Phaseolus vulgaris* (wax bean agglutinin - WBA) has a total molecular weight of 132,000 gm/mole. A glycopeptide isolated from WBA has a molecular weight of approximately 4,380 gm/mole and is composed of 123 amino acid residues and 19 sugar residues.

Figure 1: Schematic representation of the tetrameric structure of concanavalin A viewed down the crystallographic z axis. The proposed binding sites for saccharides are indicated by C.

**Classification**

One of the properties of lectins is their ability to bind to specific sugars. As a result of this property, lectins are classified according to the saccharide that they bind to. Since human red blood cells have sugar molecules on their cellular surface, it can also be said that lectins are specific for blood types. Lectins are classified into the following saccharide specificity groups: mannose, galactose*, N-acetylglucosamine, N-acetylgalactosamine*, L-fucose* and N-acetyleneuraminic acid. It is worth noting that all of these sugars are present on human cells including erythrocytes (those with a *). Since lectins cross-link to other sugar containing molecules (agglutinate), it is possible to inhibit lectins by introducing the saccharide that they are specific for (see Figure 2). Saccharides introduced for this purpose are also known as haptens (small molecules that bind to antibodies and inhibit complex formation with specific antigens).

Figure 2: Schematic representation of cell agglutination by a lectin (left) and of hapten inhibition of the agglutination (right). Cells are represented by circles with solid triangles, the latter denoting cell surface sugars. The hapten inhibitor is represented by free solid triangles.
History
Lectins were first identified by Stillmark in 1888. While investigating the toxic effects of castor bean extract (Ricinus communis) on blood, he noticed the red blood cells were being agglutinated. He isolated the material responsible for the agglutination and called it ricin. Soon afterwards, Helfin discovered that Abrus precatorius extract also caused cells to agglutinate. This agglutinin was called abrin. As a result of the discovery of these two agglutinins, some of the most basic principles of immunology were discovered such as antibody specificity and species specificity. The first lectin to be purified was concanavalin A which was isolated from the jack bean. In 1908 Landsteiner and Raubitschek established that the relative hemagglutinating activities of various lectins were very different when tested with erythrocytes from different animals and they likened this specificity to that of antibodies of animal blood serum. In 1936 Sumner and Howell reported two findings: the addition of concanavalin A to a glycogen solution caused the sugar to precipitate and erythrocyte agglutination was inhibited by cane sugar. In 1945 William Boyd discovered the blood type specific property of lectins - lectins can agglutinate erythrocytes of one blood type but not of another.

Purification and Isolation of Lectins
The most widely used way of purifying lectins is by heating the crude extract at 65°C, precipitating the lectin by ammonium sulfate, and separating the proteins by affinity chromatography. Affinity chromatography separates proteins by their binding specificity. Salt fractionation, chromatography on ion exchangers or other types of adsorbent (for example, hydroxyapatite) can also be used.

Binding of Lectins
The interaction between lectins and their receptors is analogous to that of an enzyme and its substrate: a lock and key mechanism. Like the reaction between an enzyme and its substrate, the binding of sugars to lectins is reversible and is relatively weak. If both the lectin and its compatible receptor are present, the lectin can send messages into the cell via secondary messengers or by the lectin entering the cell. The strength of the binding is dependent on the association constant and the number of unoccupied receptor sites. As lectins are capable of cross-linking, the more receptor sites available, the more clumping (agglutination) will occur.

Inhibition studies between concanavalin A and polysaccharide complexes show that polar interactions are the predominating stabilizing force between the complexes and the formation of this linkage is mostly through the non-reducing chain ends of the polysaccharides that are reacting with the lectin.

Properties and Roles of Lectins
The effects of lectins within an organism is extensive. Although there have been studies that have demonstrated their role in plants, invertebrates and other organisms, the focus here will be on their effect within the human body and a more detailed focus on their reactions with erythrocytes. Of their most notable feature, lectins are often paralleled with antibodies because of their shared characteristics of agglutinating cells,
precipitating glycoconjugates, and their ability to bind specifically and reversibly with various substrates. As such, their primary role appears to be as recognition determinants.

Lectins' apparent recognition determinant ability has prompted researchers to use them as a tool to gain an understanding into the structure of the cellular surface. Their ability to recognize "self" and "non-self" allows them to clear glycoproteins from the circulatory system, direct glycoproteins to different organelles within the cell, conduct the migration of recirculating lymphocytes from the blood stream to organs of the lymphatic system and mediate binding and phagocytosis of tumor and other types of malignant cells. For many years lectins were used in blood typing before the advent of blood type specific monoclonal antibodies. Due to their specificity and agglutinating properties, they can also be used for the isolation, purification and study of the chemical structure of carbohydrate containing polymers which include many cells within the human body.

Lectins also play a role within the immune system which is of valuable importance. Certain lectins have the ability to induce cell growth and division of lymphocytes. This 'mitogenic stimulation' is a key reaction in the body when encountered with a foreign agent. Many subpopulations of lymphocytes are specifically stimulated by particular lectins. B-lymphocytes stimulated by lectins are capable of synthesizing antibodies and T-lymphocytes may destroy foreign cells they come into contact with. The competency of an individual's immune system can be determined by using mitogenic lectins such as 

\textit{Phytolacca Americana} (pokeweed), \textit{Lens culinaris} or \textit{Lens esculenta} (lentil) or concanavalin A and examining cell proliferation or secretion of lymphokines. The effects of therapies that stimulate or suppress the immune system can also be analyzed using the same methods. The role of lectins as mitogenic agents is also used in the study of chromosomal abnormalities and the chromosomal make-up of cells. As a result of mitogenic stimulation, some biological reactions include: increase in synthesis of proteins (including RNA and DNA), acceleration of fatty acid synthesis, changes in carbohydrate metabolism, and increased uptake of small molecules. The mechanism(s) by which these responses occur is unclear, as is whether these are primary or secondary effects. Continued research is necessary to fully understand the effects of these mitogens within the immune system and the human body as a whole.

Certain lectins also have the ability to produce insulin-like reactions within the body. When this phenomena has been studied, the insulin-dependent events initiated by lectins are virtually indistinguishable from those activated by insulin. Although the mechanism of their mimicry is not completely understood, it is believed that "like recognizes like" and they bind to the carbohydrate moiety of the insulin receptor, another glycoprotein.\textsuperscript{7} Some lectins that mimic the hormone are concanavalin A, wheat germ agglutinin (WGA), wax bean agglutinin (WBA), \textit{Pisum sativum} (green pea) and \textit{Lens culinaris} (lentil). It is worth noting that many of these lectins are commonly found in the human diet. These lectins mimic insulin by triggering lipogenesis and inhibition of lipolysis in adipocytes, stimulating glucose transport and synthesis of glycogen, and promoting accumulation of lipids in adipose tissue.\textsuperscript{8} The difference between the effects
of lectins and those of insulin is the effects of the former are more persistent which results in greater deposition of fat.

This general overview of lectins presented thus far will aid in the understanding of the remaining focus on a primary property of lectins: agglutination. Due to the multiple binding sites and carbohydrate specificity of lectins they can interconnect large numbers of cells causing agglutination. This clumping of cells is the most easily detectable indication of interactions between lectins and cells. Agglutination is affected by many factors including: molecular size, number and accessibility of saccharide binding sites of the lectin, membrane fluidity, metabolic state of the cells, temperature, presence of the specific saccharide and cell concentration. Lectins demonstrate stronger binding to branched glycoconjugates or an adjacent complex of branched oligosaccharides than to unbranched chains. This binding preference is evident in their interactions with blood group antigens.

Interaction Between Blood Types and Lectins
Although there are many different blood type classifications, the discussion on the relationship between lectins and blood type will only involve the ABO system.

Erythrocytes contain surface antigens within the cell membrane which serve to trigger an immune response if they come into contact with foreign material by promoting the synthesis of antibodies (defense proteins). Antigens are glycoproteins that are composed of a ceramide attached to a base chain composed of glucose—galactose—N-acetyl-D-galactosamine—galactose—fucose. In 1953 Morgan and Watkins demonstrated that the specificity of the ABO blood type system was determined by a terminal sugar unit on the antigen. The immunodeterminants are as follows: Type O is L-fucose (of the original chain), Type A is N-acetyl-D-galactosamine and Type B is D-galactose. Lectins whose carbohydrate specificity is for D-galactose and N-acetylgalactosamine appear to be the most abundant and are present in all classes of organisms.

The ability of lectins to agglutinate erythrocytes has both positive and negative ramifications for the human body. This phytoagglutination of cells is of special importance to cancer researchers for a variety of reasons. Some lectins, such as concanavalin A, soybean agglutinin and wheat germ agglutinin, preferentially agglutinate mammalian tissue culture cells that have been introduced to oncogenic viruses or chemical carcinogens or have spontaneously transformed (see Figure 3). Malignant cancer cells are as much as 100 times more sensitive to the agglutinating effects of lectins than normal cells. This is due to the greater amount of surface sugars present on the malignant cell due to the impaired functioning of the cell. As studies of agglutination reactions of lectins with normal cells give indications as to the cellular surface components, this is also the case for malignant cells.

In the case of malignant cells, agglutination has a positive effect; once agglutinated these diseased cells are targeted for destruction by antibodies. However, agglutination does not always have such a positive effect within the body. Some of the negative
effects of this clumping include: irritable bowel syndrome, cirrhosis of the liver, blocked blood flow through the kidneys and interference with neural transmission. Since many lectins are in dietary food sources such as grains, vegetables, legumes and seafood, some researchers such as Dr. Peter D'Adamo believe that to optimize health and well-being an individual should know his/her blood type and be familiar with those foods that are incompatible with their blood type, a.k.a. cause agglutination. In this regard, D'Adamo feels that a person can avoid many common viruses and infections, lose weight as the body eliminates toxins and fats, protect against life-threatening diseases such as cancer, cardiovascular diseases, diabetes, and kidney failure, and avoid many factors that cause rapid cell deterioration.

Figure 3: Normal rat cells (left) and cells that have been transformed by a cancer inducing virus (right) after incubation for 30 minutes with 100 µg soybean agglutinin per ml.

Dietary lectins have a range of effects within the body. If the lectin reacts with a "compatible" cell this may result in beneficial effects on the digestive and absorptive efficiency of the gastrointestinal system, the immune system, presence of "good" bacteria and capacity for hormone secretion. The overall result is a positive effect on the body's metabolism. Negative effects are also dependent on blood type compatibility. The reaction between dietary lectins and the body may include the following:

- Inflammation of the digestive tract lining.
- Interference with the digestive process, causing bloating and malabsorption of nutrients.
- Reduced rate of food metabolism, therefore calories are not as efficiently burned.
- Compromised production of insulin.
- Disruption of the hormonal balance, causing edema, thyroid disorders and other conditions.
Although cooking destroys most lectins, there are some that are toxic when ingested in any form and several that cannot be degraded by the proteolytic enzymes of the gastrointestinal system. Continuing their journey intact, they may bind to the corresponding carbohydrate on mucosal cells of the small intestine and interfere with digestive, protective or secretory functions. When raw kidney beans were fed to rats, the lectins bound to the luminal surface of microvilli in the small intestine, resulting in the appearance of lesions and disruption and abnormal development of the microvilli (see Figure 4). It has yet to be determined whether intestinal permeability has been impaired as there is evidence of malabsorption of nutrients across the intestinal wall. This deficiency in absorption of nutrients could also be a result of the decrease in the activities of enzymes that break down carbohydrates and proteins which has also been observed. Researchers have yet to determine whether these metabolism altering effects are due to the insulin mimicking properties of lectins discussed previously.

Figure 4: Electron micrographs of sections through the apical regions of duodenal enterocytes from rats fed diets containing 5% raw kidney beans and 5% casein, showing severely disrupted microvilli (A), compared to 10% casein, showing normal microvilli (B).

Some of the key reactions that result from the interaction between certain foods and specific antigens are presented to give examples of the specificity of dietary lectins and blood types.

**Type A**
*Phaseolus lunatus* (lima bean) was the first lectin shown to have blood type specificity. When interacted with Type A blood, the erythrocytes agglutinate. Another Type A specific dietary lectin is *Helix pomatia* (garden snail). D'Adamo recommends that Type A women with a history of breast cancer consider introducing snails into their diet. The strong agglutinating property of this lectin to Type A blood has been demonstrated on mutated Type A cells for two of the most common forms of breast cancer. Once agglutinated, these cells are targeted for destruction. Many of the legumes, including kidney, lima, navy and garbanzo beans, have been found to cause a decrease in insulin production. An effect on the hormone regulation of insulin could lead to obesity and/or diabetes. Type As suffer strong reactions after the ingestion of tomatoes. The
lectin in tomatoes has an irritating effect on the lining of the stomach. They are also sensitive to the lectins in sweet potatoes, yams, cabbage and potatoes.

**Type B**
Foods such as corn, lentils, peanuts, sesame seeds, buckwheat and wheat encourage weight gain as a result of lectins that inhibit insulin efficiency, hamper digestive and metabolic processes, induce hypoglycemia, and inhibit liver function. Lectins such as those in wheat attach to the insulin receptors in fat cells, thus prohibiting insulin from attaching, resulting in reduced insulin efficiency. Wheat is also one of the dietary lectins that interact with immunoglobulin-E (IgE) antibodies found in the blood. These antibodies stimulate basophiles to release histamines and kinins which initiate or enhance an inflammatory response. As a result of this response, researchers question whether lectins have a role in promoting food allergies. The tomato lectin has the same negative effect within the gastrointestinal system of an individual with Type B blood as those with Type A. Rye contains a lectin that settles in the vascular system of individuals with Type B blood which may result in complications. Green vegetables, certain meats, organ meats such as liver, eggs, and low-fat dairy products encourage weight loss by aiding in efficient metabolism.

**Type O**
Wheat gluten and corn encourage weight gain in individuals with Type O blood because the erythrocytes strongly bind with the lectins of these foods which interferes with insulin efficiency and slows the metabolic rate. Other foods that impair calorie utilization include kidney beans, navy beans and lentils. Cabbage, brussel sprouts, cauliflower and mustard greens are found to inhibit thyroid production which could result in hypothyroidism. The symptoms of hypothyroidism include weight gain, fluid retention and fatigue. Foods that are compatible with Type O blood by helping to stimulate thyroid hormone production include kelp and seafood. Liver, red meat, kale, spinach and broccoli encourage efficient metabolism.

**Conclusion**
Over approximately the last hundred years lectins have been studied and found to have various properties and effects that are of great importance. Properties such as carbohydrate specificity and mitogenic stimulation have provided major insights that have broad applications within the biomedical field including advances in oncology, nutrition and genetics. With continued study and research, it can be expected that the potential of these phytoagglutinins can be further uncovered.
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Caffeine and Ephedrine: A Dangerous Pair

By:
Michelle C Geady
23 April 1999
With the struggle of weight loss so prevalent in today's society, everyone is looking for an easy solution. Enter the diet pill. With one swallow, all the worries of weight are diminished. And the effects could not be safer, especially with herbal dietary supplements, namely Metabolite 356. But Metabolite 356 contains a dangerous combination of guarana and Ma Huang, better known as caffeine and ephedrine. The drug also contains several herbs that mask some of the side effects seen from these two drugs. Even if proven effective, Metabolite 356 could prove to be fatal to your health.

For centuries, man has been plagued with an obsession of weight loss. Once able to show wealth and power, obesity has lost its ranking and has become looked upon as undesirable. Trends seem not only to affect clothing, but also their sizes; the smaller the better. Instead of a healthy and vibrant looking young athlete, the job of the role model has been passed to super-skinny supermodels. Yet, with the addition of cooperate power meetings and PTA events, traditional weight loss methods have been lost in the world of time efficiency. Unable to exercise and maintain a healthy diet, today's world has turned to easier and quicker solutions. One such solution is the diet pill and one of the newest, and perhaps most controversial of these "magical" miracle-workers, is Metabolife 356.

Metabolife 356 has taken the diet industry by storm. Along for the ride is Mike Ellis, founder and chief executive officer of Metabolife International Incorporated, and the creator of its bread-winning product.(1) Formerly a commercial real estate developer, Ellis formulated Metabolife 356 in 1995 after an earlier herbal mixture he had concocted to ease the pain his father was suffering due to bone cancer. Metabolife 356, Ellis states, is used "to help people boost their metabolism and thereby lose weight." With sales reaching over $1 million a day, Metabolife 356 proves its effectiveness and newfound popularity.

Metabolife 356 is designed to work with food, giving busy people the luxury of eating what they want or what is convenient. It is formulated to raise the body's metabolism and create a thermogenic response. This in turn burns fat. The combination of herbs found in Metabolife 356 also generates higher energy levels, which are not normally achieved through dieting and limiting caloric intake. The consumer is virtually unable to sit still, almost forced into performing some sort of athletic activity.

With the recent scare of Phen-Fen, a dangerous chemical weight loss supplement, more and more people are turning to herbal alternatives. This, however, worries doctors and other Western medical proponents. The lack of scientific experimentation about herbs and their potential side-effects has made their use controversial and often times dangerously risky. As a result of a law passed in 1994, herbal supplements are not considered drugs and can therefore not be scrutinized and regulated by the Food and Drug Administration as closely as conventional pharmaceuticals.

After the FDA banned Phen-Fen in 1997, dieters paraded to herbal alternatives to help them lose weight. Metabolife 356 contains several herbs, ranging from bee pollen to
goldenseal to ginseng and ginger. Its key component is a 728 mg mixture of guarana and Ma Huang concentrates. Prevalent in the Amazon region of South America, guarana is found to contain an immense amount of caffeine, six times as much as is found in the coffee plant.(2) In America, caffeine has become the most popular drug, easily abused for its stimulating effects.

Ma Huang is Chinese ephedrine.(3) While Metabolife 356 contains a relatively small amount of ephedrine, the drug, which raises the heart rate and is used in China to treat bronchial asthma, is still under intense questioning. Physicians are unsure of its safety, especially if used to lose weight and in combination with other herbs. Ephedrine can also be found in drugs that boost energy and is a key ingredient of methamphetamines. Its diasteriomer, pseudoephedrine, can be found in several over the counter cold remedy aids.

In several studies, Metabolife 356 has proven to be safe and effective. Yet, it is also proven that both caffeine and ephedrine have side-effects that cannot be ignored as more and more consumers are negatively affected by the weight loss drug. This combination of caffeine and ephedrine is also speculated to be dangerous and can produce such side effects as heart palpitations, hypertension, and digestive disorders.

A Closer Look at Metabolife 356's Ingredients

Guarana

Caffeine-yielding plants were discovered as early as 600,000 to 700,000 years ago during the early Stone Age. Searching for food, the people of the Paleolithic era chewed on the bark, leaves, and seeds of several plants, and were struck by the mood-altering effects of the caffeine laden plant. First ground and eaten as a paste, it was soon discovered that boiling the plant in hot water produced more enhanced effects, as it is possible to extract more caffeine at a higher temperature. Eventually, this crude form of caffeine was cultivated to alleviate stress, elevate mood, prolong and sharpen attention, and prevent fatigue.

Guarana was first discovered and used by the Brazilian Indian tribes. Before the invasion of the white man in the Amazon jungle, the Indians of the Tapajos harvested the tough seeds of the guarana plant and baked it into a hard paste. During a hard day of labor, the paste, softened with hot water provided an indispensable amount of energy. Other forms of caffeine beverages also became popular. Tea was discovered in China and enjoyed by Shen Nung, Chinese Emperor in 2700 BC. Eleventh century Arabians put the coffee plant to use in their beverage. And in 1519, Spanish conquistadors were treated to a chocolate drink by Emperor Montezuma upon their arrival to the New World.

Caffeine is a naturally occurring substituent of guarana that can be extracted and used for its own purposes. It is a mild stimulant that affects people differently according to their sensitivity and past usage of the drug. Deemed the most widely used and popular drug of this century, caffeine is used to improve performance of simple tasks that require attention rather than memory. It delays a decrease in performance due to exhaustion or boredom. In projects that call for memory, caffeine has been shown to speed up performance, but hamper quality, creating numerous mistakes.
The scientific name for caffeine is 3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione.
Its chemical formula is C8H10N4O2.

Figure 1. A molecule of Caffeine

Because caffeine is soluble in water and easily passes through membranes, it can be found in any part of the body that contains water. When taken orally, it is easily and quickly absorbed from the stomach, passing into the bloodstream which transports it to all the organs of the body, including the organs of a developing fetus.
Caffeine travels first to the liver, where it is broken down into metabolites through a process called metabolism. These metabolites are eventually excreted through the urine, leaving no traces of caffeine in the bloodstream. Its main line of attack is against adenosine, a chemical that supplies energy to all the cells of the body, including brain cells. Increased adenosine levels can cause lethargy and sleep. It can also cause dilation of blood vessels, diminishment in the contraction of the gastrointestinal muscles, lowering of the heart rate, and decrease in blood pressure.

Adenosine also inhibits the release of neurotransmitters, the chemicals that carry messages from one cell to another, by binding itself to the cell surface. Because they are so similar in structure, caffeine can also bind itself to these cells, prohibiting adenosine. The result is the ability of neurotransmitters to fire messages more rapidly. Caffeine also lowers the amount of adenosine in the bloodstream, allowing the muscles and organs to work at an accelerated speed.

There are several acute side effects of caffeine use. High blood pressure is caused when the heart is strained to pump blood through constricted blood cells. Caffeine increases the blood pressure, taking it to sometimes dangerously high levels. Caffeine can also affect the heart rate. It may initially decrease the heart rate, but will always slightly increase it. Caffeine may also be associated with arrhythmia, or abnormalities in the hearts rhythm. Palpitations are a form of heart arrhythmia in which the heart may skip a beat, perhaps causing heart failure and eventual death.

Caffeine also dilates the blood vessels in the brain, increasing blood flow. Because it heightens the sensitivity of the area of the brain that responds to the level of carbon dioxide in the blood, respiration is also increased. Caffeine does, however, promote deeper breathing because it strengthens the action of the diaphragm. It is therefore a useful treatment in those with bronchial ailments.

Because it is so identical to adenine and guanine, two building blocks of DNA, in structure, caffeine can also interfere with the successful duplication of DNA. These errors in structure can lead to tumors, cancer, and genetic defects.

Caffeine works in the body to increase the rate in which food is converted to useable energy. If taken between meals, it causes fats to be transferred to the bloodstream, where, as fatty acids, it can be used as energy. Caffeine also stimulates the temperature regulating centers of the body, raising body temperature. Therefore, more calories are burned even when at rest.

Caffeine also works negatively in the digestive system. When caffeine enters the stomach, it increases the amount of hydrochloric acid in the stomach almost by 200%. This rapid increase raises the risk of ulcer development in the stomach and also decreased peristaltic movement. Therefore, the caffeine remains longer in the stomach and small intestine, hampering the body's ability to adsorb important nutrients, namely iron, calcium, magnesium, and sodium.

Because caffeine is so popular, it is also very easily abused. Caffeine dependency can occur quickly, but it is easy to rid the body of it. Caffeine withdrawal may include symptoms such as sever headache, nausea, restlessness, and difficulty sleeping. These conditions usually only occur for five days.

Ma Huang
For over 200 years, the Chinese have used Ma Huang, ephedrine, in treating bronchial asthma.\(^4\) It has also been used to improve blood circulation, relieve coughing fits, and break fevers. Today, ephedrine and its diasteriomer pseudoephedrine are used in several over the counter pain relievers, cold remedies, bronchodilators, and dietary supplements. Ma Huang was first discovered in the Chinese mountains. Literally meaning "ask-for-trouble" its use has provided a controversy over its safety. It's chemical formula is C10H15NO, and its structure is closely related to that of the methamphetamines (C10H15N). In fact, ephedrine is the key ingredient in the street drugs "Ecstasy" and "Speed."

**Ephedrine**

- **Formula:** CHNO
- **Molecular Weight:** 165.23
- **CAS Registry Number:** 299-42-3
- **Chemical Structure:**

![Ephedrine Chemical Structure](image)

Figures 2 and 3. A Molecule of Ephedrine and Methamphetamine

Acute effects of methamphetamine use includes increased heart rate, increased blood sugar levels, muscle tension, and a false sense of well-being. The extra energy given through their use is borrowed from the body's reserve. When the drug's action has worn off, which usually is 6-8 hours after initiation, the body pays in severe fatigue and depression. This creates the desire and need for more of the drug.\(^5\)

The side effects of ephedrine are similar, although not as severe. Ephedrine over-stimulates the heart muscle causing palpitations and hypertension.\(^7\) It raises the blood pressure considerably and induces a general state of nervous sensitivity. Because ephedrine is a prompt and effective decongestant, with the ability to clear nasal and bronchial passages leading to the lungs, it has been, and is still used, in the treatment of hayfever, asthma, and emphysema.

Research has also shown that ephedrine affects the central nervous system. It is given to patients who are in alcohol- or drug-induced comas to counteract the depressant effects of the drug. Ephedrine is also a vasoconstrictor, meaning that it narrows the blood vessels. Because it also increases the heart rate, this can provide a danger for those suffering from hypertension, but a treatment for those with hypotension.

Ephedrine is also addictive. Prolonged use can produce excessive perspiration, weakness of the body, insomnia, anxiety, and possible schizophrenia. It has been said that this drug can be used to promote energy and aid in weight loss. Due to the dangers of the drug, these uses of ephedrine should be avoided, specifically in conjunction with caffeine.\(^8\)

**Bee Pollen**
Mythically, the fountain of youth resides in Florida, but many people feel a trip to this place of mystery is unnecessary. Bee pollen is said to reverse the aging process. In some, it promotes a feeling of youth and rejuvenation. Composed mainly of proteins, the building blocks of amino acids, bee pollen contains many substances needed to maintain life. As a constituent of Metabolife 356, bee pollen works to improve the appetite and increase fitness levels.

Bee pollen has also been proven to regulate the hormonal and digestive systems. It also normalizes the activity of the intestines and aids in building resistance against diseases. Known for its healing powers, bee pollen is enriched in aspartic acid, an amino acid capable of stimulating the glands and enforcing mental and physical well-being. Possible side-effects of bee pollen include itching, dizziness, and difficulty in swallowing.

Ginseng

Ginseng was first discovered in the Chinese mountains in 55AD. It credits its name, Ren Shen, or Man's plant, to the father of two overambitious boys. Having just been taught to hunt, the boys went off on a trip of their own, but were suddenly caught by an early winter. Trapped because of excessive snowfall, the boys ran out of food, and were forced to live on a sweet and juicy root. They began to feel more energetic, and ate more and more until one of the boys' nose began to bleed. Eating the plant in moderation, the boys were able to sustain life during the winter and returned to a very surprised village. When questioned about the root, they said only that it resembled a man.

Ginseng is used to treat chronic fatigue, shortness of breath, profuse sweating, lethargy, and chest and abdominal distention. It can also be used to stimulate the appetite, enhance athletic ability, and dispel palpitations accompanied with anxiety. Denoted the "King of Herbs", ginseng contains vitamins A, E, and B12, thiamin, riboflavin, niacin, calcium, iron, phosphorus, sodium, silicon, potassium, magnesium, manganese, sulfur, and tin.

The Chinese use ginseng as a tonic to tone up the original energy of the body. Medically, it is an effective anti-shock herb. It increases red blood cells, produces adrenocortical hormones, decreases blood sugar, and reduces blood fat. It should be taken during signs of energy deficiency, a weak pulse, a lack of appetite, hypertension, and insomnia. Due to its powerful effects, only small doses should be taken. Tea and turnips must be avoided while taking ginseng because they reduce its effectiveness.

Ginger

Called Gan Jiang in China, wild ginger root is effective in treating coldness, nausea, vomiting, phlegm accompanied coughing, bloating, and spasms. Ginger root also aids in appetite stimulation and digestion. It can ease the pain of compressed joints and spinal injuries and act as a rubefacient, reddening the skin by increasing blood flow to a given area.

Ginger is used all over the world. In Canada, a preparation of the plant serves as a remedy for heart palpitations. For some Indian tribes, ginger is used to regulate the menstrual cycle. The early settlers used the plant to ease intestinal and stomach gas, and
to break fevers. In Tibet, ginger is used to stimulate the energies of one who is debilitated. And in Japan, ginger root is used as a pain reliever and appetite stimulant.

Lecithin

Lecithin regulates metabolism and breaks down fat and cholesterol, preventing it from adhering to artery walls. It can be found in bee pollen and works in conjunction glucomannan to distribute body weight and maintain a healthy nervous system. It also contains tipotropic substances, which prevents water retention.

Damiana leaf

Metabolife 356 also contains damiana leaf, which has stimulating properties. It has been used for nervousness, weakness, fatigue, and hormone balance. It can also increase sperm count in males and strengthen the egg of a female. It is especially beneficial for an exhausted state of the body and of the vital organs of the system, providing necessary energy and strength. Children can also use this safe herb as a mild laxative.

Sarsaparilla root

Found in Moonseed and Spikenard, sarsaparilla root contains berberine, an herb long employed in the treatment of various chronic illnesses. It is an effective diuretic, laxative, and is also useful in treating ailments from tuberculosis, swollen lymph glands of the neck, and rheumatic and arthritic diseases. Its tea is recommended as a tonic and perspiration inducer, aiding in breaking fevers.

Sarsaparilla root was once used by the American Indians to relieve backache and muscle pain. The Shawnee used it to treat gas pains, coughs, asthma, and chest pains. Women were instructed to use it to make childbirth easier and less painful. It has also been used to heal broken bones and disinfecting open cuts and wounds. Relatively safe, Texas Sarsaparilla, or Moonseed, produces poisonous berries that can cause death by rapid pulse, severe vomiting, and purging. In Metabolife 356, sarsaparilla root is used as an appetite stimulant, helping the product work to its full potential.

Goldenseal

Goldenseal was once very important to the American Indians. Named for its root, goldenseal produces a golden yellow dye, which was used as a ceremonial and war paint. It brought upon the bearer luck and safety. The herb itself is a contrast to this belief. In large doses, the alkaloids that cause the plant's drug action are poisonous.

Goldenseal is effective for inflammation of the eyes, mouth ulcers, cancer, tuberculosis, and edema. It also stops bleeding and hemorrhaging, and can be utilized as an antiseptic. In combination with other herbs, goldenseal is mainly responsible for enhancing their potency and enforcing their healing properties.

Nettles leaf

During the Bronze Age, nettle was used as a crude linen and nicknamed the "textile plant." To keep warm at night, Roman legions would cluster under piles of nettle bushes. The nettle leaf contains silica, a glass-like particle which can puncture the skin of a human, animal, or insect. The plant injects formic acid into it "victim", which causes a
burning sensation under the skin. Boiling can extract the silica, and the healing powers of the plant can begin.

Believed to be a cure for everything from baldness to tuberculosis, nettle can be used to control bleeding, asthma, and skin problems. The plant contains alkaloids that neutralize uric acid in the body to help alleviate rheumatism. It is also a good counterinflammant, meaning that it irritates the skin of an inflamed area, thereby increasing blood flow to the area and decreasing swelling. Nettle is also extremely rich in iron, replacing any that may be lost to caffeine use. Nettle is also enriched with vitamins A, C, E, F, P, and minerals sodium, copper, manganese, chromium, and zinc. Other than iron, nettle also contains first-class calcium and vitamin D.

**Gotu Kola**

Also known as "Food for the Brain", gotu kola combats stress and anxiety to improve mental and physical power. It is best used after a nervous breakdown to help rebuild energy reserves and enliven the brain cells. It is employed to relieve high blood pressure, mental fatigue, senility, and helps the body defend itself against toxins. Fortified with vitamins A, G, and K, gotu kola is also comprised of magnesium.

**Spirulina Algae**

Spirulina is a natural food supplement that helps to balance the diet. Considered one of nature's foods, spirulina is easily digestible and quickly assimilated. It strengthens the body when it is weak, providing vitamins and minerals that satisfy the hidden hunger the body craves when it is not getting enough essential nutrition. After an acute illness and during a chronic illness, spirulina builds the vitality the body needs, and also purifies the blood as it nourishes the cells of the body. Rich in protein and fatty acids, spirulina is also high in vitamins A, B, B12, C, E, iron, magnesium, phosphorus, calcium, potassium, and sodium. Spirulina is basically composed of all the necessary nutrients required by the body.

**Royal Jelly**

Not much is known about royal jelly, except that it is used to treat cases of fatigue and depression. It works in conjunction with ginseng to regulate poor digestion and increase the absorption of food in the intestines.

Many of the herbs found in Metabolife 356 aid in its purpose. They help to increase the metabolism, aid in the increase of appetite, and provide a natural surge of energy. Yet, these herbs are also used to treat many of the side effects from caffeine and ephedrine, specifically hypertension, heart arrhythmia, and nervous anxiety. While currently, research in this field has proved inconclusive, more research into the herbal world is necessary to provide safe alternatives. The most effective way to safely lose weight is to maintain a healthy diet and participate in a regular exercise program. Although the diet pill will produce fast and pleasing results, it's irreversible side effects may slowly be destroying that new-found health.
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(1) www.metabolife.com
ESTRADIOL

Keri Hoyer
April 23, 1999
Estradiol is the major estrogen produced in mammals. It is used as a hormone replacement therapy for both males and females. It may be instrumental in fighting the late onset of many types of cancer. Although there are many possible side effects, Estradiol is the most common hormone used in medicine. This paper investigates the basic properties, crystalline structure, bond distances, bond angles and endocyclic torsion angles of Estradiol.

Estradiol is the accepted IUPAC name for Estra-1,3,5(10)-triene-3,17-diol. Its molecular formula is C$_{18}$H$_{24}$O$_{2}$, with a molecular weight of 272.386. The melting point of Estradiol is 223°C. Estradiol has many other commonly used names as well as several variants and their derivatives (1 and 2). Estradiol is a steroidal compound which is reproduced in the sex glands of mammals and transported to the ovaries and uterus, where their effects are produced. It is a major estrogen secreted by the premenopausal ovary. Estradiol falls into the estrogenic hormone class which induces the development of secondary female sexual characteristics and controls the uterine cycle (4). Its effects are seen in the development of the embryo as it develops primary female characteristics and during puberty when it regulates gene transcription and protein synthesis for secondary female characteristics. It also induces the production of gonotropins, which in turn induce ovulation (6). Estradiol can be isolated from pregnancy urine (1).
Estradiol is often given as a hormone replacement therapy in menopausal and postmenopausal women or for menstrual disorders. It is also used in the management or breast cancer in menopausal and postmenopausal women and can even be used for the management of prostate and breast cancer as well as osteoporosis. It may be administered by mouth, as a topical cream, subcutaneously by injection or through skin patches. Estradiol is absorbed from the gastrointestinal tract and through the skin and mucous membranes. It becomes partly bound to plasma proteins and is rapidly metabolized. It is excreted in the urine mostly as sulfate and glucuronide esters and in small amounts as unchanged Estradiol (3).

NTP reports limited evidence of Estradiol being a carcinogen in humans, but sufficient evidence was found by the EPA’s genetic toxicology program that it may have carcinogenic effects in other animals. When used in studies with experimental carcinogenic, neoplasticigenic, tumorigenic and teratogenic data, it was confirmed to be a carcinogen promoter (2). Estradiol may have adverse effects on the cardiovascular system, including greater incidence of venous thrombo-embolisms. This was verified when the U.S. coronary drug project treated men with previous heart problems with 2.5 mg a day of Estradiol. Analysis of this project also showed an increase in gall bladder disease in this same group of men. There is one report of hypersensitivity in a patient who had received an injection. This patient later had an
anaphylactic reaction. One other report suggests possible adverse affects on the nervous system in a 57 year old woman with a history of migraine headaches (3).

Estradiol is "creamy white, odorless, colorless, hygroscopic crystals or crystalline powder." It is insoluble in water. Its solubility in alcohol is 1 in 28, in ether it is 1 in 150 and in chloroform it is 1 in 435. It is also soluble in acetone, dioxan and slightly soluble in vegetable oil. It must be stored in air tight containers and protected from light. It has a pH between 3.5 and 6.5 (3).

A crystallographic analysis was done on an ENRAF NONIUS CAD-4 diffractometer using a type of monochromated radiation scan technique. "The crystals showed systematic absences in reflections consistent with the orthohombic space group P2₁₂₁₂₁. The refined lattice parameters, \( a = 12.589(2), b = 16.274(5), \) and \( c = 23.535(6) \) A, were obtained by a least squares fit to a set of 20 measured values for 25 reflections in the interval of 16<20<22. The calculated density is \( D_x = 1.184 \) g/cm." A stereo plot of the molecule shows bond distances, bond angles and endocyclic torsion angles. The torsion angles for the ring junctions are listed in the table. There were no unusual values found for the bond lengths and angles in the non-hydrogen atoms. The C-H bond distances range from 0.94 to 1.14 A and the two O-H bond distances range from 0.86 to 0.94 A (5).

---

Stereo Plot of Molecule
Torsion Angles for Ring Junctions

A / B
C1-C10-C5-C6  -176.0
C4-C5-C10-C9  -175.8

B / C
C10-C9-C8-C14  174.8
C7-C8-C9-C11  -179.3

C/D
C12-C13-C14-C15  167.3
C8-C14-C13-C17  -178.8

Intramolecular Dimensions

Bond Distances; Range: 0.002 - 0.004

A)
Bond Angles; Range: 0.2 - 0.3

B)

C12-C13-C17 = 114.6
C14-C13-C18 = 113.8
C17-C13-C18 = 109.7
O17-C17-C13 = 114.2
Endocyclic Torsion Angles; Range: 0.3 - 0.4

C\textsubscript{S} (C13) = 4.9
13n - envelope

C\textsubscript{2}(C7-C8) = 9.4
7, 8n - half chair
C\textsubscript{S} (C8) = 13.0
8n - sofa

"Analysis of the conformational data showed that exocyclic non-bonded interactions play a decisive role in determining overall steroid conformation. This was found to be especially true with interactions found across the A/C and B/D -bay regions. Most of the conformational flexibility
of the steroid backbone is located in the B ring region.” These B rings have been observed in chair, half chair and sofa conformations. The half chair conformation is stabilized by the interactions between the 1 and 11 Carbons. The sofa conformation, however, is less stable because the 1 and 10 and 9 and 11 Carbons are eclipsed. Three other crystal structures are also known that are intermediate between the half chair and sofa conformations. The reason for the three different B ring conformations seen above may be the difference in the intermolecular interactions (5).
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MODERATION! MODERATION! AND MODERATION!
Aspartame, an artificial sweetener, has many uses, both commercially and in the home. When consumed in moderate amounts it is a very safe food substitute. However, like most things, if abused, by consuming it in large quantities, it can prove harmful. When aspartame is broken down, its different components are either stored in various parts of the body or eliminated.

Aspartame was discovered, by accident, in 1965. While working at the Searle Research labs in Skokie, Illinois, James Schlatter was trying to synthesize an inhibitor of the gastrointestinal secretory hormone, gastrine, that could be used in treating ulcers. At the time, Schlatter was recrystallizing L-aspartyl-L-phenylalanine methyl ester from ethanol. When the mixture was bumped and spilled on his hand. Consequently, when he licked his fingers to pick up a piece of weighing paper, he discovered the remarkable sweet taste of this dipeptide ester.

Dipeptide ester, later named aspartame, is a white crystalline powder completely without odor. This characteristic makes it a good artificial sweetener that can be used as a sugar substitute. Aspartame is composed of “L-phenylalanine, which is bitter and L-aspartic acid, which is flat.”\(^2\) When combined properly, the two amino acids that form aspartame make a product that is as sweet as sugar. Aspartame is a stereoisomer where the L form is sweet to the taste and the D form is bitter.

![Chemical structure of aspartame]

There are two basic categories of sweeteners, natural and synthetic sweeteners. Natural sweeteners include honey, fruit, corn syrup, and sorghum, while artificial or synthetic sweeteners are saccharine, cyclamates, aspartame. One hundred and fifty to two hundred times sweeter than sucrose, aspartame was introduced to the American public in the early 1980s. The FDA approval was obtained 1981 for use in any foods and as a tabletop sweetener, under the names of NutraSweet\(^8\) and Equal\(^8\). By 1983, aspartame had received the go-ahead to be used in carbonated beverages such as Diet Coke. Aspartame has many commercial applications; it is used to sweeten low calorie foods such as puddings, gelatins, and yogurts. A one-gram package of aspartame has four calories, equal in strength to two teaspoons of sugar, which contains thirty-two calories.\(^4\)
Aspartame is used both commercially and in the home, though not without certain limitations. When baked at high temperatures for a long time, aspartame loses its sweetness. However, it can only be used at low temperature such as: added during last few minutes of heating, sweetening soft drinks, teas, coffee, puddings, gelatins, yogurts, on fresh fruit, and cereal.

How much of aspartame can one consumes before any detrimental effects will be seen? Studies have been conducted on animals to determine and set the toxicity levels for humans. The World Heath Organization Technical Report Series 653 evaluated the toxicity in animal studies and several humans studies. The no-adverse-effect level, based on animal studies, was found to be 4 mg/kg. However, according to the Monsanto Company, which holds the patent on aspartame, for an adult person the level is 50 mg/kg. Furthermore, the acceptable daily intake (ADI) established by the FDA is based on comprehensive animal and human studies.

It is calculated that an average adult of about 132lb could consume 3,000mg per day, (equivalent to 85 packets of aspartame or 18 cans of diet soda) every day of his or her life and still not reach toxic levels in body. For example, even if a person were to eat a wide variety of foods every day that contain aspartame, it would be impossible to consume 3000mg in one day, much less everyday. These are some of the food products that contain aspartame and the amount:

- 12oz of carbonate beverage (180mg)
- One 4oz gelatin desert (95.0mg)
- An 8oz powdered drink (120 mg)
- 6oz of hot chocolate (50.0mg)
- A 4oz of ice cream (50.0mg)
- A 4oz of pudding desert (25.0mg)
- 6oz of fruit drink (10.0% juice) (70.0mg)
- One bar of breath mint (1.50mg)
- A stick of gum (6.00-8.00mg)

If one ate all of these for snacks the total aspartame intake would be 598.5mg (equivalent to 0.600g), which is only a fifth of the ADI.

The first synthesis of aspartame was in 1966. There is more than way to synthesize aspartame. A common method is non-regiospecific ring opening of N-protected aspartic anhydride (1). This leads to a mixture of products from which aspartame must be separated. Below is the schematic for this alternate method starting with aspartic acid:

![Diagram](image_url)
However, by starting with L-aspartic acid N-thiocarboxyanhydride(6), an attack by phenylalanine methyl ester (5) occurs regiospecifically at a favored carbonyl to give a high yield of product. Moreover, it also has an added advantage in that it does not have to be separated from a mixture of products. Below is the schematic for this alternate method starting with aspartic acid:

The ethyl xanthate (3) was reacted with a mixture of aqueous sodium hydroxide and methanol to give thiourethane (4). When the thiourethane in ethyl acetate was treated with PBr$_3$, a mildly exothermic reaction took place, and white crystal of L-aspartic acid N-thiocarboxyanhydride(6), hydrolized aspartame is formed.
After, a person has ingested aspartame, it is metabolized by the body to decompose into three component parts: methanol, phenylalanine, and aspartic acid.  

![Chemical structure of aspartame and its metabolites]

The methanol absorbed into the body and utilized in the same way as when it is obtained from fruit or juice. When the compound reaches the liver, the first process occurs is the oxidation reaction to convert of methanol to formaldehyde, by the alcohol dehydrogenase. Then formic acid is converted to carbon dioxide and expired out of the body.

\[
\text{CH}_3\text{OH} + \text{NAD}^+ \xrightarrow{\text{alcohol dehydrogenase}} \text{H-C-} + \text{NADH} + \text{H}^+
\]

\[
\text{H-C-} \xrightarrow{\text{catalase}} \text{H-C-} + \text{OH}
\]

\[
\text{H-C-} + \text{H}_2\text{O}_2 \xrightarrow{\text{peroxide}} \text{CO}_2 + 2\text{H}_2\text{O}
\]

The amount of methanol that produced by aspartame is relatively so small compared to natural foods. For example, a glass of tomato juice has six times as much of methanol as an equivalent amount of diet soda.

Aspartic acid is one of the several amino acids present naturally in many foods.
No differentiation is made, by the body between aspartic acid in asparame and that found in foods. Therefore, this component is metabolized by the body and waste products are then excreted in a normal fashion.

\[
\begin{align*}
\text{Aspartic acid} & \quad \text{N}\text{H}_2\text{C} - \text{C} - \text{C}_2\text{H}_2 - \text{C} - \text{C} - \text{O}_2 \\
\text{alanine} & \quad \text{CH}_3 - \text{CH} - \text{C} - \text{O}_2 + \text{CO}_2 \\
\text{alanine} & \quad \text{CH}_3 - \text{CH} - \text{C} - \text{O}_2 + \text{N}\text{H}_2\text{C} - \text{C} - \text{C}_2\text{H}_2 - \text{C} - \text{C} - \text{O}_2 \\
\text{2-oxoglutaric acid} & \quad \text{OH} \\
\text{pyruvic acid} & \quad \text{NH}_2 \\
\text{glutamic acid} & \quad \text{C} - \text{H} - \text{C}_2\text{H}_2 - \text{C} - \text{C} - \text{C} - \text{O}_2
\end{align*}
\]

The aspartic acid proceeds through a decarboxylation reaction, and gives off alanine. The alanine reacts with 2-oxoglutaric acid to form pyruvic acid. The pyruvic acid formed, thus proceeds to the Krebs Cycle to drive the formation ATP.

Half of the aspartame molecule is composed of L-phenylalanine. This component mainly is metabolized to tyrosine.

\[
\begin{align*}
\text{Phenylalanine} & \quad \text{NH}_2\text{C} - \text{CH} - \text{CH} - \text{COOH} \\
\text{O}_2 & \quad \text{NH}_2 \\
\text{Tyrosine} & \quad \text{OH} - \text{C}_2\text{H}_5 - \text{CH} - \text{COOH} \\
\text{4-Hydroxyphenylpyruvate} & \quad \text{O}
\end{align*}
\]

Phenylalanine is another essential amino acid, is a part of the building block that make up protein. The amounts of these component is very small compared to the amounts obtained from the daily food intake. For example, 3 ounces of chicken contains 1026 milligrams of phenylalanine, and twelve ounces of diet soda contains about 90 to 100 milligrams of phenylalanine.
The body acquires and utilizes aspartame in one of two ways; it is either broken down and hydrolyzed into its component parts by the proteolytic and hydrolytic enzymes found in the lumen of the intestine, or mucosal cells may absorb it directly through peptide transport mechanisms. Once aspartame is within the mucosal cell, it undergoes hydrolysis into aspartate, phenylalanine, and methanol. Regardless of which method, is used by the body to incorporate aspartame into the system and begin its break down. When large doses of aspartame are consumed, it breaks down its constituent parts, which are released into the portal blood where they need to be utilized by the body or eliminated as a waste product.

Upon aspartame’s breakdown and subsequent metabolism by the body, methanol is released into the circulatory system. This is because aspartame is a methyl ester. Therefore, any potential for toxicity must be viewed with the substance’s methanol content taken into complete consideration when conducting studies on how the body utilizes aspartame and the biological effects the compound has on the body. This said and acknowledged, leads researchers into having to consider each on of the components of aspartame, and the blood concentrations of each, separately and as a whole. The concentrations of aspartate, phenylalanine, and methanol need to be noticeably elevated if toxic effects are to be produced and observed.

Since aspartame’s introduction, researchers have been studying its biological effects on different age groups, those with chronic diseases such as diabetes, pregnant and nursing women and the effects it may have on the unborn fetus. In one study of dieting 17 to 21 year olds, lasting fourteen weeks, the subjects level of immunoreactive glucagon rose in both the control group and the experimental group even though weight loss continued, albeit at a slower rate the second seven weeks then during the first seven weeks. From this study, and other like it, it was concluded that “aspartame has no synergistic action with caloric restrictions to stimulate glucagon release. The same can be said for insulin, because aspartic acid and phenylalanine likewise are suggested to stimulate insulin secretions from the beta cells of the pancreas.”

Monosaccharide and disaccharide, or refined carbohydrates, are the most often restricted food items in a diabetic’s diet, whether they are insulin dependent or non-insulin dependent. Because it is generally believed that if diabetics wish to maintain the recommended level of blood glucose, they cannot consume glucose and glucose containing disaccharides, and simple carbohydrates. Sweeteners are extremely difficult for one of two reasons; people have an acquired craving for sweet tasting things are something all humans are born with. Therefore, this is a group of people for whom artificial sweeteners are a necessity if they wish to have a wide selection and choice of sweet, palatable foods. Unfortunately, even though diabetics are one of the largest groups who frequently, and habitually consume the artificial sweetener aspartame, there is very little published data on aspartame’s biological effects on blood glucose levels.

Aspartame is a dipeptid that is hydrolyzed to its amino acids in the gastrointestinal tract and therefore it is possible that can affect the secretions of insulin, growth hormone and glucagon. These hormones can then, in turn, affect the body’s
blood glucose levels. However, when taken into account the amounts given over the specified period of time for ingestion, as indicated in the studies, the secretion of the above mentioned hormones would not be noticeably altered. Studies have also provided data that cite that the ingestion of aspartame does not effect the body's insulin secretion. Instead, the data leads researchers to believe that if a person consumes a sweet substance that is devoid of any significant amount of calories, such as aspartame, insulin is not secreted.

According to Pitkin and Baker the consumption of aspartame by both pregnant and nursing women does not have any adverse effect on either the unborn fetus or the nursing infant. One reason for this is that aspartame's constituent amino acid, aspartate, phenylalanine to an extent, and methanol do not cross the placental barrier. This is because the placenta is, in essence, impermeable to aspartate, and phenylalanine with can only cross if there are extremely high levels in the mother's system.

One must keep in mind at all times when investigating the biological effects of drugs or compounds on humans, that at some point, at some level, all compounds are toxic to the body. The question is, at what dosage and for how long, over a period of time, will toxicity occur. If one chooses to use sweeteners, nutritive or non-nutritive, then a balanced diet should be taken into consideration and sweeteners used in moderation. The various choices of non-nutritive sweeteners help one select the product that is individual best suited to meet one's needs. Research into alternative sweeteners and those currently on the marked should continue. In the best interest of those persons, i.e. diabetics who are in need of these substance.
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CAFFEINE

April 23, 1999

By: Eric J. Noland
CHM 236
Abstract

Caffeine is the world's most popular drug. It is found in all types of foods from beverages to desserts. 80% of the world's population consumes caffeine daily. The use of caffeine far exceeds that of any other commonly used drugs such as nicotine and alcohol. Caffeine has been around for centuries and can be found in tea and other drinks. Caffeine has been known to have many possible health benefits, but as always there are adverse side effects to health.
Introduction

Products using plants containing caffeine have been used for centuries. Not until the 17th century did caffeine products become a world wide phenomenon. Previously, in ancient times, caffeine products were used only in particular locations around the world. Historical records show coffee plants were transported from Ethiopia to Arabia in the 15th century. Then by the 16th century the idea of the coffee being brewed was common practice in the Islamic world. The Dutch were the first to bring and cultivate coffee beans out of Arabia. And from there the idea spread throughout the rest of the world.(5)

Today caffeine-containing products are still used throughout the world, but now there is more information about the chemical caffeine. The chemical composition, chemistry, negative side effects, and positive side effects.

How it Works

Caffeine containing products come in many various forms: pop, coffee, tea, chocolate, etc.. Many of the products have different forms of caffeine.

One of the most common forms of caffeine 1,3,7-Trimethylxaline. Trimethylxaline is one of a family of methylated xanthine, or methylxalines.(3) Methylxanthises are alkaloids. There are a few different methylxaline compounds: Theophyline, theobromine, etc.. All of these are found in different caffeine containing products. The difference between the compound is only seen by the presence of methyl groups in two positions of the chemical structure. These groups are easily oxidized to uric acid and other methyl uric acids. Caffeine is anhydrous or contains one molecule of water. So when storing caffeine it must be in a tight dry container.

. When identifying caffeine as a product caffeine will appear as a white crystal or powder. It will have no smell, and taste slightly bitter. The melting point of caffeine should be in the range of 235-238 degrees Celsius.(1) The product should be soluble in chloroform, partially soluble
in water and ethanol, and hardly soluble in ether anhydride. (3) An example of what caffeine should look like on the u.v. spectrum is shown on fig. 2.

**Health Effects**

The process by which caffeine metabolizes in the human body is fairly straightforward (fig. 3). The process of biotransformation is performed by the liver, and is regulated by the cytochrome p-450 enzyme system which includes demethylation of caffeine (1,3,7-trimethylxanthine). This reaction results in three dimethylxaline metabolites, paraxanthine (1,7-dimethylxanthine), theobromine (3,7-dimethylxanthine), and theophylline (1,3-dimethylxanthine) (see fig.). In most adults the caffeine is transformed into the above three, and less than 2% would be found in urine unchanged. (5)

The major product produced in the body from caffeine is paraxanthine (1,7-dimethylxanthine) in humans. About 84% of product will be paraxanthine, theobromine at 12%, and theophylline for 4%.

**Side Effects/Criticism**

Within the past few years there has been a growing concern for possible dangers to health from caffeine intake. Caffeine is infamous for having displeasing side effects. Some of these effects include irritability, nervousness, restless, headache, nausea, and even vomiting. (9)

A major concern of possible dangers from caffeine abuse found was the possible effects on birth weight of newborns. Some studies have shown a decrease in birth weight with high levels of caffeine intake by pregnant mothers. (6)

One study done in a Belgrade hospital during 1992 and 1993. 1032 women were interviewed and asked to estimate the amount of caffeine consumed during pregnancy. Among the 86% of women who consumed caffeine, of an amount of 71mg/day or more, there was found to be a significant reduction in birth weight. (6)
Alkaloid, $\text{C}_8\text{H}_{10}\text{N}_4\text{O}_2$, purified from plants of Coffea genus, Rubiaceae.

**Fig 2.**

UV spectrum

- Retention time: 2.75 min
- Optimum wavelength: 210 nm
- Sensitiveness: 1.48 ng
- Absorbance / 100 ng: 0.4738 abs.sec
Figure 2.1. Caffeine and its dimethylated metabolites in humans
NOTE: arrow widths indicate relative proportions of the metabolites in plasma.
Another concern to think about when consuming caffeine is the diuretic or dehydrating effect. For example if you drink a cup of coffee or cola, after a few hours you will urinate roughly 50% of the liquid you take in with the beverage.(4)

Even though the toxicity of caffeine is low, there have been fatal overdoses resulting in misuse of caffeine. The main reasons death would occur in a caffeine overdose are convulsions, cardiac and arrhythmia’s. A lethal blood serum of caffeine in humans fatally poisoned range between 79-710mg. In one fatality an overdose occurred with a caffeine concentration of 1560mg.(9)

Side Effects

While there are many dangers there are also many benefits of caffeine if caution is taken. Probably the most well known benefit is the increased alertness. Studies done at Walter Reed Army Institution of Research done for the military. 150mg, 300mg, and 600mg of caffeine were administered to volunteers, after 48 hours of sleep deprivation the volunteers were tested for the next 12 hours. The volunteers given the 600mg dose had improved performances. They showed object alertness, and self-ratings of mood to the same extent that 20mg of amphetamine had shown in a comparable study. The caffeine however had fewer negative side effects.(7)

The other benefits gained by caffeine consumption have been seen by athletes. One benefit to an athlete’s performance is increased endurance. Caffeine has been known to boost endurance and adrenaline secretion.(8)

Many marathon runners swear by there caffeine to help push their bodies to there limits. One research team studied six healthy young men. While the participants worked out on exercise bicycles there blood pressure was monitored. After the workout the participants were each given a double espresso. The participants performed the same task again, the second time the participants hearts were found to use less oxygenate pump more blood a, and a lower blood pressure was monitored.(2)
Conclusion
Caffeine has had its place in the world's diet for centuries, and caffeine is guaranteed to be in the world's diet for many more years. Caffeine while it may have its negative side effects, if used properly it can be very useful in athletics and other professional areas.
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BETA CAROTENE: A Promise for the Future?

Structural and Synthetic Overview with Insights on Present and Future Applications.

Cristina Pace
April 23rd 1999
Abstract—Beta carotene (BC) is an important carotenoid, which finds applications in the food industry and as a nontoxic precursor of vitamin A. Recently it has also been studied as a possible treatment for different types of cancer, including prostate and lung cancer. Even though some lung cancer studies were inconclusive, the potential of BC makes this carotenoid still worth studying, especially how it can be commercially synthesized and how it works.

BC is one of the most common of over 500 carotenoids\(^1\), which can be found naturally not only in carrots, but also in many fruits and vegetables, such as squash, sweet potatoes, pumpkins, spinach, broccoli, apricots and cantaloupe. Interest in this particular carotenoid arises from the fact that it can be transformed by the body into vitamin A, which is an important antioxidant that protects body tissues from harmful oxidation reactions and is, among other things, essential for good vision. BC, however, proves to be a much better alternative, since, unlike vitamin A, it is not toxic to the liver—where it is stored in the body—even when taken in high quantities\(^2\). Besides its role as a precursor of vitamin A, BC seems to affect cell growth and maturation and is therefore studied as a potential chemopreventive or chemopostponing agent in aging, immune deficiency, senile cataracts and in several types of cancer.

This report will examine the structure of BC, its natural and commercial syntheses and the research involved with it. Some historical background will be mentioned throughout the report when appropriate. First the early problems with determining the crystalline structure of BC will be discussed, followed by the accepted BC structure provided by Sterling. Then, after a brief description of the biosynthesis of BC, its commercial synthesis will be discussed in detail, looking at the various ways of synthesizing BC and the success rate of each. In the end, insights about the ongoing research on BC will be presented, looking at past, present and future.

After Taylor's first attempts to resolve the molecular structure of BC in 1937 were inconclusive, no further experiments were performed in that direction until 1963, when Sterling was finally able to determine the crystal structure of BC\(^3\). Chemical evidence at the time indicated that BC (all-trans-β-carotene), was a planar, all-trans, polyene chain with conjugated double bonds, attached methyl groups, and a β-ionone ring at each end, also with attached methyl groups. Since both chemical and X-ray evidence suggested that the molecule had a center of symmetry, Sterling focused only on one half on the molecule, neglecting the hydrogens.

Through his experiments, Sterling came up with roughly the same unit-cell dimensions that Taylor had found, which are \(a = 7\,55,\ b = 9\,51,\ c = 24\,8\) Å, and \(β = 105°3°\). However, he made an interesting discovery. Studying the results of the electron density distribution, he realized that some of the peaks were better explained with a \(s\text{-}cis\) configuration at the \(C(7)\) and \(C(6)\) bond. Even though the structure postulated on that basis still did not work, it led in the right direction and, with further adjustments on the least square program, the chain and ring of the half molecule were finally recognizable. The \(s\text{-}cis\) configuration was not only confirmed by the low \(R\) value obtained (0.19) and the short length of the \(C(6)\)-\(C(5)\) bond (135 Å), but also by the fact that the angles about the atoms at each end of the \(C(6)\)-\(C(5)\) bond came up to a total 360°.

Despite the large standard deviations in most of the interatomic distances (0.03 Å) and the deviant values for some of those distances in the ring, the experimental values of interatomic distances in the chain were fairly reasonable. If those intrachain distances are accepted, a new interesting relationship is found: the shorter and larger bond lengths in BC are clearly alternating with little sign of bonds of mixed nature. Putting all information together, Sterling suggested the following structure for BC, which is still accepted today:
Once the structure was clear, the next step was to see how BC was synthesized in nature, in order to better understand how it could be best synthesized commercially as well. There is no doubt now that BC is synthesized naturally in the chloroplasts of vascular plants together with chlorophyll a, and studies suggest that it also plays an integral part in the photosynthetic process that takes place in the chloroplasts. It is derived from geranylgeranyl diphosphate (GGPP) through phytoene formation, desaturation and cyclisation. Two possible cyclisation pathways have been studied and, while considerable evidence has been found to support the cyclisation of lycopene (2; \(\psi,\psi\)-carotene) with \(\gamma\)-carotene (4; \(\beta,\psi\)-carotene) as an intermediate, cyclisation of neurosporene (1; 7,8-dihydro- \(\psi,\psi\)-carotene) to \(\beta\)-zeacarotene (3; 7,8-dihydro- \(\beta,\psi\)-carotene) and then to \(\gamma\)-carotene through dehydration is still circumstantial. This, however, does not exclude the possibility that both pathways could contribute to the ultimate synthesis of BC.

Back to GGPP, key intermediates in its synthesis are farnesyl diphosphate (FPP), isopentenyl diphosphate (IPP) and mevalonate (MVA). However, there are conflicting views about whether the early intermediates, especially MVA, are autonomously formed within the chloroplasts or whether they have to be imported. Findings from \(^1\text{H}\)- and \(^{13}\text{C}\)-NMR analyses of MVA and glycine labeling suggest that MVA is both partly synthesized within the chloroplasts and partly imported from outside, while IPP and the other intermediates are all synthesized within the chloroplasts. In fact, in part GGPP is biosynthesized via the condensation of FPP derived from exogenous MVA and endogenous IPP, and in part it is also synthesized from endogenous MVA derived from acetyl-CoA via glycine.
Figure 3: biosynthesis of BC—general overview

In the end, the biosynthesis of BC also involves certain feedback inhibition mechanisms that are linked with the formation of phytoene. Studies show that a decrease in the combined formation of phytoene and squalene triggers feedback inhibition before the formation of FPP. This can slow down the overall rate of the process by inhibiting one or more of the enzymes between MVA and FPP.

Even though the mechanisms involved in the biosynthesis of BC are still not fully understood, BC has been commercially synthesized by Hoffmann-La-Roche (HLR) since 1954 and by BASF AG since 1972. In the beginning, commercial synthesis of BC was primarily aimed at the food industry to use in the direct coloration of food, like in margarine, or in the synthesis of canthaxanthin, another important carotenoid that gives egg yolk a more appealing orange-red color and also enhances resistance to disease and improves the shelf life and hatchability of eggs. However, recently BC has also boomed as a substituent for vitamin A and as a biological antioxidant. Both HLR and BASF, which at least up to 1991 still had the preserve on BC, started synthesizing it by using carbon-carbon linkage methods that were already proven in vitamin A chemistry. At HLR they first tried that through Grignard syntheses and enolether condensations, while at BASF they chose ethynylations and Wittig olefination syntheses.
Comparing the two different approaches, HLR started with C_{14} aldehyde, which, through two enolether condensations, gave C_{19} aldehyde. Two moles of C_{19} aldehyde were then linked together to form BC after partial hydrogenation and acid-catalyzed elimination of two moles of water. BASF, on the other hand, started by either linking two moles of the C_{15} phosphonium salt precursor for vitamin A to the symmetrical C_{10} dialdehyde, or by linking retinal to the C_{20} phosphonium salt obtainable from vitamin A. After thermal isomerization, both routes provided crystalline all-E BC in yields above 80%. The higher yield and the simpler process, which makes multistage synthesis more economical, proved that Wittig olefination reactions were the best methods for the synthesis of BC.

Figure 4: Top—synthesis of BC via enolether condensations; bottom—syntheses of BC via Wittig reactions

Once the Wittig reaction was established to be the best synthesis method for BC, HLR started investigating that process, too. The major problem with Wittig olefination was the formation of triphenylphosphine oxide, which, on an industrial production scale, had to be recycled by reduction to triphenylphosphine. Thus, HLR looked at another olefination reaction reported by Julia and co-workers in 1973, which involved sulfones. By running the reaction in aqueous tetrahydrofuran or 1,2-dimethoxyethane with 25% ammonia or organic bases, such as diethylamine, BC was formed in yields up to 90%, thus proving more effective than the standard
Wittig olefination reaction. In addition, while Wittig reactions gave only all-E BC, the sulfone olefination reactions produced also Z-isomers, which have caught great interest in the last few years as possible anticarcenogenic agents.

The first route investigated by HLR involved two moles of C_{15} sulfone and one mole of C_{10} dialdehyde. The sulfone (1a) was deprotonated by butyllithium and then C_{10} dialdehyde (2) was added at -60°C. After in situ acetylation, treatment with aqueous sodium hydroxide and crystallization, the (all-E)-11,11'-bis [(p-chloro-phenyl)-sulfonyl]-β,β-carotene (5a) was formed in 85% yield. Reduction by an excess of sodium dithionite / 25% ammonia in 1,2-dimethoxyethane gave, after isomerization and crystallization, BC (6a) in about 90% yield [hplc: 90% by weight (all-E), 3% (9Z)].

Figure 5: synthesis of BC involving C_{15} sulfone
The second route involved one mole of C_{20} sulfone and one of C_{20} aldehyde. The sulfone (9) was prepared from vitamin A acetate and then coupled with retinal (10). The intermediate (11) that formed was then directly converted into the C_{40}-sulfone (12) and \textsuperscript{1}H NMR spectroscopy revealed the expected all-E or 15Z configuration. The reduction of the C_{40}-sulfone by dithionite took much longer than that of the disulfone of the previous route and crude BC was obtained in about 80% yield.

**Figure 6: synthesis of BC involving C_{20} sulfone**

Even though the commercial synthesis of BC is still largely used for the coloration of foods, at present the main interest in BC lies primarily in relationship to its functions as a provitamin A supplement and as an anticarcinogenic agent. As already mentioned, BC is in part naturally transformed by the body into vitamin A and, unlike vitamin A, it is not toxic to the liver even in high doses. Therefore it potentially becomes the perfect supplement for all the body’s needs of vitamin A. This is particularly true in developing countries, in which the populations are at high risk of vitamin A deficiency and mostly rely on dietary provitamin A carotenoids, such as BC, to meet their vitamin A needs. At the same time, since after Dorogokuplyya et al first announced in 1974 that BC minimizes skin cancer in mice\textsuperscript{10}, a new branch of research on BC has developed in that regard and a number of studies have been performed on the possible role of BC as an anticarcinogenic agent.
Provitamin A supplement:

Even though, for its nontoxicity, BC was considered a safe alternative vitamin A supplement, one main problem was to see if it was also an effective replacement for all the major functions of vitamin A. One of those functions, for instance, was to increase iron absorption and prevent iron-deficiency anemia, which is a widespread problem especially in those countries that base their diet primarily on foods like rice, wheat and corn, which have high contents of inhibitors of iron absorption. Therefore, solubility experiments as well as human absorption studies were performed comparing the iron absorption of rice, wheat, and corn alone, with vitamin A, or with BC. Results showed that both vitamin A and BC increase iron absorption considerably and, in addition, BC also prevents the inhibitory effect of polyphenols on iron absorption, so that when coffee was added to the diet, it did not significantly decrease iron absorption as it should be.

Once the expectations on BC as a vitamin A supplement were positively confirmed, one of the main concerns of nutritionists was how cooking and processing would affect its bioavailability. Because of the correlation between plasma BC response and bioavailability, Garcia-Casal and co-workers examined the plasma BC response to raw vs. processed carrots and spinach in women, and the findings suggest that heating does not neutralize the enhanced BC uptake associated with consuming commercially processed vegetables, compared to raw vegetables. However, heating promotes isomerization from all-trans to cis, and some results suggest that the cis BC isomers have less provitamin A activity than the all-trans isomers.

Anticarcenogenic agent:

The effect that BC seemed to have on cell growth and maturation suggested that it might be an effective anticarcenogenic agent. According to a widely regarded hypothesis, free radicals are the most important DNA-damaging agents in mutagenesis and carcinogenesis and all-trans BC is believed to function as a lipid-soluble trap for free radicals, thus slowing down or even stopping the spreading of cancer. In support of that hypothesis, various studies showed that, for instance, BC completely blocked the formation of liver tumors in animals, reduced the risk of prostate cancer in people with low initial BC levels in the blood, protected the skin against UVA damage, and significantly increased the activity of natural killer cells in elderly men, which, in turn, may have had beneficial effects on the prevention of cancer and viral infections. On the other hand, other studies showed puzzling results that may even suggest that BC increases the risk of cancer. The Beta Carotene and Retinol Efficacy Trial (CARET), for instance, was suspended after four years because study results showed 28% more lung cancers and 17% more deaths in patients—smokers—taking BC and vitamin A.

Before jumping to hasty conclusions and dooming BC forever, a few considerations must be made. First of all, many studies on lung cancer, like the CARET study, were performed on smoker patients and those results cannot be generalized to nonsmoker patients as well. Apparently, BC supplements seem to slightly increase the risk of lung cancer in smoker patients, and it has been hypothesized that abnormal smoke-damaged lung cells are kept alive by BC when they should have died off, which does not happen with non-smoker patients. In general, however, even though BC is not toxic, one important side effect is that it depletes the body of vitamin E and this, in turn, can increase the chances of getting cancer, unless the diet is adequately balanced with the necessary intake of vitamin E. Moreover, the synthetic BC is all-E and lacks the 9Z- isomer that is also present in natural BC, which makes quite a difference,
since many studies suggest that the 9Z-, together with other Z- isomers, may actually be a more potent antioxidant than the traditional all-E isomer.

One of those studies on the potential involvement of Z- isomers of BC as better anticarcenogenic agents was undertaken by Doering and co-workers and was based on the hypothesis that if all-E BC acts as a trap for free radicals, a diradical generated by twisting all-E BC 90° C about one of its double bonds should be even more effective. Experimenting on the kinematics, Arrhenius parameters, and thermochemistry of the cis isomers of BC, they found that when all-E BC is heated at 37° C, 13Z- and 15Z isomers are generated unavoidably. This, together with their kinematic and thermodynamic competency, suggests that both 13Z- and 15Z isomers are the true anticarcenogenic agents in place of the conventionally accepted all-E BC. In addition, the 9Z-isomer is another potentially interesting anticarcenogenic agent. In fact, all these isomers [(9Z), (13Z), (15Z)] show very little steric hindrance, in contrast to the remaining Z-isomers [(7Z), (11Z)], which explains why they are potentially better candidates.

In conclusion, since 1963, when Sterling first revealed the crystalline structure of BC, a lot has been discovered about this molecule: where and how it is synthesized in nature, how it can be effectively synthesized commercially in yields up to about 90%, and that it can be used in the coloration of foods, as a vitamin A supplement, and possibly as an anticarcenogenic agent. Research on BC as a vitamin A supplement has given great results so far, showing that BC can effectively and safely take care of the body’s needs for vitamin A, as well as satisfy all the main functions of vitamin A, like in the case of iron absorption. Unfortunately, research on BC as an anticarcenogenic agent has not shown the same positive results and has raised many new questions, like why it increases the risk of lung cancer in smoker patients and why the synthetic supplement is not as effective as expected. However, recent studies from different sources suggest a new promising line of research in that regard, which would focus on team work of BC and vitamin E to limit the risk of cancer, and also on Z- isomers of BC, especially 9Z, 13Z and 15Z, which appear to be better anticarcenogenic agents, in contrast to the all-E BC, which seems to work better as a vitamin A precursor.
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Paxil

An Antidepressant

By:

Mike Papamatheakis
Abstract

Paxil is an antidepressant which selectively inhibits Serotonin re-uptake. Paxil's history, physical properties, side effects, and case studies will be briefly presented. A brief discussion on how Serotonin plays a role in depression will also be presented. Synthesis of Paxil will be evaluated in great detail.

Physical Properties

Paxil, otherwise known as Paroxetine Hydrochloride is an antidepressant which selectively inhibits 5HT re-uptake. Since its initial launching in 1991, Paxil is the seventeenth best selling prescription drug\(^1\). It is a hydrochloride salt of phenylpiperdene compound otherwise known as (\(-\))\(^-\)-trans-4R-(4fluorophenyl)-3S-[(3,4-hemihydrate)]\(^2\). Paxil has an empirical formula of \(\text{C}_{19}\text{H}_{20}\text{FNO}_3\) HCL 1/2 H\(_2\)O. Paroxetine Hydrochloride is an odorless, off-white powder which has a melting Point of 120 to 138 degrees Celsius. Paroxetine Hydrochloride has a solubility of 5.4mg/ml in water. Paroxetine Hydrochlorides structural formula is illustrated in fig 1.

Fig 1

![Chemical Structure of Paroxetine Hydrochloride](attachment:image.png)
Paroxetine Hydrochloride is used in the treatment of depression. The antidepressant action of Paroxetine Hydrochloride and the effectiveness of Obsessive Compulsive Disorder (OCD) and Panic Disorder (PD) is linked to potentiation of serotonergic activity in the Central Nervous System (CNS) resulting from inhibition of neuronal reuptake of Serotonin (5-hydroxy-triptamine, 5HT). It is noted that Paxil may be used if you have four of the following eight symptoms: change in appetite, change in sleep, retardation, loss of interest in sex drive, increase of fatigue, feelings of guilt and worthlessness, slowed thinking or impaired concentration, and suicide attempts. Animal studies showed that Paroxetine Hydrochloride is a potent and highly selective inhibitor of neuronal Serotonin re-uptake which has very weak effects on Norepinephrine and neuronal reuptake. Therefore, Paroxetine Hydrochloride’s primary effect is on Serotonin, not on other functionality’s in the body.

Paroxetine Hydrochloride is completely absorbed after an oral dosage. Approximately thirty four percent of a thirty milligram oral dose was excreted in the urine with 2 percent of the parent compound and sixty two percent of the metabolized compound. Paroxetine Hydrochloride has a relatively long half life: two days after a single dose and eight days after multiple doses. In a study in which male subjects (n=15) received Paroxetine Hydrochloride for thirty days. Paroxetine Hydrochloride concentrations were achieved by approximately ten days for most of the subjects. The Paroxetine spread throughout the entire body including the central nervous system (CNS), although approximately ninety five percent of Paroxetine Hydrochloride is bound to plasma protein.
If an overdose of Paroxetine Hydrochloride occurs, some possible symptoms could include: nausea, vomiting, drowsiness, sinus techycardia, and dilated pupils. There have been no reports of coma or convulsions and no deaths were reported following overdose of Paroxetine Hydrochloride alone.  

5HT (Serotonin) plays a very serious role in depression. Serotonin has a structural formula depicted in figure 2.

Fig 2

![Serotonin, 5-HT](image)

The view that Serotonin plays a distinct role in depression is supported from the clinical and experimental evidence that Serotonin is involved in the regulation of sleep, vigilance, memory, learning, feeding, and sexual behavior, all of which are signs of severe depression.

Serotonin produces its psychological effects by activating one or more subtypes of the of 5HT receptor. The 5HT subtypes which are primarily involved in depression are still uncertain, but, further testing in rats have shown that chronic antidepressant treatment results in a hypersensitivity of postsynaptic 5HT1a receptors and a hypersensitivity of the presynaptic 5HT1a receptors. SSRIs (Selective Serotonin Re-uptake Inhibitors) inhibit the uptake of 5HT into the
nerve terminal by binding to the Impramine binding site. (see figure 3). This intern affects the biological response of the system.

**Case study**

In a six week double blind experiment, one hundred and twenty outpatients suffering major depression were randomly assigned treatment with Paroxetine Hydrochloride, Impramine, or placebo. The patients had to be a minimum of eighteen years of age and could not pose any suicide risks. The dosage could be increased to a maximum of fifty milligrams of Paroxetine Hydrochloride or two hundred and seventy five milligrams of Impramine. The dosage would be increased depending on the side effects or response of the patient. Measurements were achieved by the HAMD scale, the Montgomery-Asberg Depression scale (MADRS), the Clinical Global Impression scale (CGA), the Raskin Depression scale, the Covi Anxiety scale, and the 56 item Symptom Checklist (SCL-56).

The treatment groups were similar in respect to age, sex, psychiatric history, and scores. Figure
shows the percentage difference of all the trials. This experiment showed superiority of Paroxetine Hydrochloride over both placebo and Impramine. Paroxetine Hydrochloride was most often associated with drowsiness, fatigue, and gastrointestinal side affects. While Impramine caused dry mouth constipation and urinary symptoms. One patient was excused due to liver problems.

Fig 4

![Graph showing percentage change with different treatments](image)

Synthesis

The synthesis of Paroxetine Hydrochloride is as follows:
When carbon 14 formaldehyde was added to 4 (4-fluorophenyl) -1 methyl 1,2,5,6- tetrahydropyradine (2) it forced a recemic mixture of (3) (only one enantiomer shown). A primary alcohol was forced to add to the cycloalkene as shown in (3). This first step must be handled with great care due to (4) (4-fluorophenyl) - 1-methyl-1,2,5,6-tetrahydropyradine being a suspected toxin.

Simple hydogenation was followed using H₂/Pd. This reaction would attack a double bond because a double bond is highly strained. This reaction had a choice of which double bond to attack. It can attack a double bond on the benzene ring or it can attack a double bond on the heterocyclic ring. If the benzene ring is attacked, the molecule will lose stability because benzene is very stable with three double bonds. If it attacks the heterocyclic double bond, the molecule will be more stable because there will be an increase of stability in
Work Cited

the molecule. Therefore, the heterocyclic double bond is attacked.

Benzenesulphonyl chloride (PHSO2Cl), under strong basic conditions produced the sulphonate ester. This was treated with sesamol followed by chromatographic purification which gave a fifty-fifty recemic mixture of (6). Next the (−)-trans isomer of (6) was obtained by using its (t) tartaric salt. This step was than double checked for its optical activity. Demethylation of the free base (6) was performed using vinyl chloroformate which gave (1) otherwise known as Paroxetine Hydrochloride.

Paroxetine Hydrochloride is a safe and effective treatment for depression. The synthesis for Paroxetine Hydrochloride is relatively simple but requires great care and preparation. It can be said that Paroxetine Hydrochloride is very effective compared to other Selective Serotonin Re-uptake Inhibitors (SSRI). (e.g Impramine or Fluoxetine)
Paradise Valley Community College

Diabetes Etiology & Prevention

Thank you, Dr. Mancini, for teaching the process.

A chain
Gly—Ile—Val—Glu—Gln—Cys
Cys—Ala
Leu
Tyr
Gln
Leu
Glu
Asn
Tyr

B chain
Phe—Val—Asn—Gln—His—Leu—Cys
Gly—Val—Leu—His—Ser
Ala—Leu—Tyr—Leu—Val—Cys—S—S—Cys
Gly

Ala—Lys—Pro—Thr—Tyr—Phe—Phe—Gly—Arg—Glu

Organic Chemistry 236
Dawn Swanson
Monday, April 19, 1999
Paradise Valley Community College

Diabetes Etiology & Prevention

Thank You Dr. Mancini for Teaching the Process

A chain
Gly—Ile—Val—Glu—Gln—Cys—Cys—Ala

B chain

Ala—Lys—Pro—Thr—Tyr—Phe—Phe—Gly—Arg—Glu

Organic Chemistry 236
Dawn Swanson
Monday, April 19, 1999
Abstract: Though Diabetes I & II are considered completely different diseases, (Diabetes I being an autoimmune disease, the other a kind of wearing out of the body) the end result is the same—high blood sugar with the Beta cells in the Islets of Langerhorns (Pancreas) unable to transport insulin into the cells. In the end, they both produce the same devastating physical results. This paper discusses the mechanisms of diabetes, etiology, and theories which may prevent both Diabetes I & II.

Diabetes

In the United States:

- Diabetes is the fourth leading cause of death, killing more than 162,000 people each year.
- The mortality rate of patients with insulin-dependent diabetes increases dramatically after 15 years of disease duration.
- 665,000 new cases of diabetes are diagnosed annually.
- 123,000 children and 1.4 million adults have type I diabetes.
- Type I is the third most prevalent severe chronic disease of childhood after asthma and mental retardation.
- Diabetes is the leading cause of new cases of blindness in people between 20 and 70 years of age.
- Globally, 10-20 million people suffer from Diabetes I.
- The incidence of Diabetes I increases 3-5% per year\(^1\).

Virtually every major organ system in the body is damaged by diabetes. Complications include blindness (nearly 39,000 Americans lose their sight every year). Kidney failure (1 out of 3 people with insulin-dependent diabetes develop Nephropathy and need kidney transplants). Heart disease—Arteriosclerosis (diabetes can cause arteriosclerosis which leads to heart disease, gangrene, and loss of extremities). People with diabetes are 2-4 times more likely to have heart disease than the general population, stroke, amputation of extremities and loss of nerve sensation—early loss of teeth, high-risk pregnancies and babies born with birth defects.

The cost to the health care system for the medical treatment of diabetes and its complications is in excess of $100 billion per year. Both Diabetes I & II are on a dangerously high increase around the world.

In identical twin studies, an identical twin has almost a 100% chance of developing adult onset diabetes if the other twin develops the disease. For juvenile diabetes, the identical twin has approximately 33% chance of developing the same disease. However, because diabetes II is an autoimmune disease, the identical twin can and typically develops one of the many autoimmune diseases, particularly hyper/hypothyroidism. Gene therapy researchers know the genes
responsible for diabetes, however these same genes are also responsible for other autoimmune diseases, including Graves disease, Lupus, Rheumatoid Arthritis, Multiple Sclerosis, and more.

The Etiology of Diabetes

Diabetes II noninsulin dependant, is typically an adult onset diabetes. There are 500,000 new cases diagnosed in the United States each year with 90 percent involving obese individuals. Three cultures now claim an average of 50% adult population with children also developing the disease as early as 9 years of age. These societies are: The Pima Indians in Arizona, Australian Aborigines, and the Malays in Singapore².

In this type, insulin levels are elevated and with Beta cells seriously weakened, they are sluggish or unable to transport glucose the cells. Treatment involves weight loss, dietary restriction which lowers blood sugar and drugs that lower plasma glucose concentrations, primarily by reducing glucose syntheses and release within the liver.

Dan Benyshek, C. Johnston, C Hart, and Dr. J. Martin, biomedical anthropologists at Arizona State University, have studied these populations extensively, and specifically the Eriksson, U.J., & Sweene, 1993 study, which shows protein deprivation in utero, which is then followed in child and adulthood by a normal diet, combined with little exercise can typically lead to Diabetes II.

Why? Protein deprivation causes the liver, for a lifetime, to make higher than normal blood sugar, with the islet of Langerhorns (Pancreas) producing weak and fewer Beta cells. Beta cells produce insulin. After birth, when the diet becomes normal, compounded by an already high blood sugar, the Beta cells are overworked and unable to handle excessive glucose. When diet does not improve the onset of adult diabetes can be seriously delayed or not at all.

To illustrate: imagine a small army--Beta cells--fighting a large army--high blood sugar produced by the liver. Feed the blood sugar side, and blood sugar easily wins, because the Beta cells are weak from overwork, fatigued, therefore, they tend to exhaust and/or die quicker. However, keep the blood sugar low simply by diet and exercise, and the Beta cells stand a better chance of doing their job.

Juvenile Diabetes (Type I) is now understood as an autoimmune illness, the body's own white blood cells, (particularly T-cells) which normally fight infection, turn on "self". In diabetes II, these white cells target the cells which produce insulin (the beta cells of the islets). Over time, so many of these cells are lost that there is a lack of insulin and diabetes subsequently develops³.

Glucose transport in cells cannot occur without insulin, even after a meal that is rich in glucose, therefore tissues are glucose-starved.

Insulin is a peptide hormone released proportionally to the amount of glucose in the blood. Insulin binds to receptor proteins on the cell membrane which leads to the activation of the receptor and attaches to the phosphate groups to intracellular enzymes. Phosphorylation of enzymes produce primary and secondary effects within the cell. Insulin receptors are present in cell membranes that are insulin dependent and enter these cells by facilitated diffusion. Other
cells which require insulin, yet do not have receptor sites are located in the brain, kidneys, lining of the digestive tract, and red blood cells.

Proteins offer diverse functions: as enzymes or hormones. Proteins catalyze and regulate the reactions that occur in the body, as in muscles and tendons, they are the outer covering of skin and hair, and in hemoglobins, they transfer oxygenate, as antibodies, they provide animals with protection against disease.

In cells, enzymes move substances in and out of the cell by specificity for the substrates and their products. One such combination is labeled Lock and Key hypothesis. The enzyme is the lock and the substrate, the key. They have a shape that is geometrically complementary, which literally works with the key opening the door of the cell’s wall to enter. This site on the cell’s wall is called the active site and cells have hundreds, if not thousands, of active sites each site specifically ready for it’s substrate. Nonequivalent forces, Van der Walls, electrostatic forces, hydrogen bonding, and hydrophobic interactions beckon the substrate into the enzyme. Even though enzymes are stereospecific, they can vary in their geometric specificity, while one enzyme will fit with one compound, others will accept a variety with similar groups.

Therefore, when glucose is abundant, insulin is secreted, which stimulates operation to support growth and the establishment of carbohydrate (glycogen) and lipid (triglyceride) reserves. Glucose utilization activates key enzymes—second messengers that are involved in the initial steps of glycolysis, and stimulates amino acid absorption and protein synthesis.

Glucose (from amino acids, glycogen, fatty acids in adipose tissues) is used for energy from the cellular level up. The liver breaks down glycogen metabolized for energy in the skeletal muscle fibers or to release glucose into the bloodstream. “The size and structure of glycogen beautifully suit its function as reserve carbohydrate for animals. Its size makes it too large to diffuse across cell membranes; thus glycogen remains inside the cell where it is needed as an energy source.”

“Animals store energy as fats (triacylglycerols) as well as glycogen. Fats, because they are more highly reduced, are capable of furnishing much more energy. The metabolism of a typical fatty acid, for example, liberates more than twice as much energy per carbon as glucose or glycogen. Glucose (from glycogen is readily available and is highly water soluble. Glucose, as a result, diffuses rapidly through the aqueous medium of the cell and serves as an ideal source of “ready energy.” Long-chain fatty acids, by contrast, are almost insoluble in water and their concentration inside the cell could never be very high. They would be a poor source of energy if the cell were in an energy pinch. On the other hand, fatty acids (as triacylglycerols) because of their caloric richness are an excellent energy reservoir for long-term energy storage.”

Auto Immunity is better understood with a brief description of how the immune system works. In simplistic terms, the immune system recognizes anything foreign to the body—viruses, a splinter, toxins, transplant organs, including the injection of human insulin. The immune system then launches a war against this foreign object, to prevent the virus from spreading or killing an organ and/or body, or causing an infection.
Specifically, the recognition system of the body malfunctions. It sees “self” or Beta cells in the case of diabetes, specifically Beta cells GAD (Glutamic acid decarboxylase) protein on the surface of the Beta cells as an invader. The invader is similar to a known toxin or virus, therefore called “molecular mimicry.” This is comparable to mistaken identity of identical twins in that they look alike, physically. The immune system activates the B and T cells and together they recognize and begin secreting cytokines that promote more B cell activation, and division, accelerate plasma cell formation which produces the antibodies. These antibodies bind to receptor sites of the antigen and kill the virus/toxin/cell.

B cells perform two functions, one to make antibodies, and two, B cells with memories. They keep books, they never forget. Once a war has been waged, fifty years later, if the same virus enters the body, the B cells quickly remember and use the same antibodies to kill the invader.

Interleukins are chemical messengers that coordinate the defenses against the viral infection. Interleukins tell the cells and their neighbors what to do. Some interleukins suppress the immune response, others enhance. The suppression interleukines tell the cell when an immune attack is finished, the other, when to begin. One interleukine currently under study is IL-4 which researchers believes suppresses the immune system involved in the destruction of insulin-producing cells. In a study of twins where one had diabetes, and the other did not, they found the twin without diabetes had large amounts of IL-4, whereas the twin who developed diabetes had absolutely none.

One last important feature to understanding diabetes is Cell Death. Cells have a built in feature, regulated by protein growth factors, how many times they will divide. These secreted signal proteins bind to cell-surface receptors, and signal the cell for growth and division. If deprived of this signal, the cell cycle stops and though the cell can remain in remission for days, weeks or even years before dividing again, a start signal must be sent, otherwise the cell will die. In a Diabetes 1, the Beta cells when is the process of dying can take weeks to years to die. This process is known as the “Honeymoon.”

I believe that it is possible in protein deprived pregnant mothers, and possibly anemic pregnant mothers (discussed later) that the protein growth factor seriously underestimates the number of cell divisions necessary for a lifetime of pancreatic Beta cell health.

External Factors contributing to Diabetes

Measles, Mumps & Rubella Immunizations (MMR). Researchers believe this is a case of molecular mimicry. The MMR, especially the measles arm resembles the GAD protein on the cell’s outer membrane. Autoantibodies are found before other antibodies, including insulin antibodies in animals developing diabetes. GAD exists in two isoforms and are based on molecular weight—GAD65 and GAD67. Three peptides of GAD65p6, 14, 15 and two peptides of GAD67p2, 3 elicit strong T cell responses. I.E. when an antigen such as the Measles virus is injected, these particular GAD proteins are most similar and T-cells specifically target them. Therefore if a protective coat were to be produced to protect GAD proteins, it might be helpful to target these GAD-proteins specifically.
It is interesting to note that a high incidence of juvenile diabetes develops after late, or repeated MMR injections. Typically, this group is among health professionals or mother’s to be who believe they may not have had the MMR injections or disease and therefore want to protect their baby against exposure while in utero. (Note that in utero exposure to German measles will cause juvenile diabetes more than 40%, along with other devastating problems.) Rather than run a titer, they receive the injections and within a matter of months enter the Honeymoon stage of juvenile diabetes.

In Science News, The Dark Side of Immunizations, J. Barthelow Classen, a physician who heads Classen Immunotherapies in Baltimore examines the incidence of diabetes not necessarily based on the amount of MMR’s, but when immunizations are given. Babies typically begin immunizations at 2 months, when their immune systems are believed adequate to initiate an immune response. However, Classen wonders if perhaps the juvenile-onset diabetes take root because a baby’s immune system is asleep at the switch early on, where viruses are passed from mother to child at birth which inflames the insulin producing islet cells, and waiting for two months, allows a child with a tendency toward a disease more time to become more susceptible later on when other challenges come along like German measles, Strep, and the Coxsackie virus which cause a polio-like infection, all of which attack the Pancreas’ beta cells.

In other animal studies, the absence of contact with naturally occurring viruses increase the risk of diabetes, which suggest that certain viruses’ protect against the disease, and/or prime the immune system. In Sweden, tuberculosis vaccines were given at birth until 1975. At that time, the Swedes gave children other immunizations, but not at birth: Diabetes rose dramatically after 1975. Studies show that unvaccinated children report fewer cases of diabetes and other autoimmune disease.

**Coxsackie Virus and Strep**

These two virus’ are to juvenile diabetes as carcinogens are to cancer. Both virus are on the serious increase around the world. Wherever there is an outbreak of the Coxsackie-virus; an outbreak of diabetes is sure to follow. Both Strep and Coxsackie range from mild symptoms to severe. Fever, headache, muscle aches, mild sore throat, abdominal discomfort or nausea. The mechanisms are the same as the German measles, attacking the GAD protein.

Persistent low grade infections such as these when passed from mother to child in utero can cause Diabetes 1.

**Cortisol Hormone:**

Cortisol, stimulated by ACTH (adrenocorticotropic hormone, also called hydrocortisone) accelerate glucose synthesis and glycogen formation, especially within the liver. Adipose tissue replaces fatty acids into the blood, and other tissues break down fatty acids and proteins instead of glucose. This is an anti-inflammatory effect which inhibits white-blood cell activity. On the short term this is effective to slow allergic reactions, and slow histamine release which would promote inflammation.
The flip side is that it suppresses the immune system, lipid reserves are mobilized and peripheral proteins are broken down, i.e. higher blood sugar with possibly weaker/sluggish Beta cells and/or other enzymes.

Exaggerated long term stress where high blood sugars are prevalent, stresses the Beta cells and already overworked immune system. During periods of stress, if any of the external causes of diabetes occur, this could easily cause diabetes.

**Cow’s milk**

*Bovine Serum Albumin, and Nitrosamine* \(^9,10\)

There is considerable belief that cow’s milk, especially introduced early in an infant’s diet causes diabetes, and in fact, diabetic children were significantly less likely to have been breastfed than nondiabetic children. Diabetics were 60% more likely to have had an early exposure to cow’s milk than nondiabetic children.

\[
\begin{align*}
(CH_3)_2NH + HCl + NaNO_2 \rightarrow (CH_3)_2N\cdot N=O \\
H_2O 
\end{align*}
\]

If indeed this is real, then researchers believe there is a molecular mimicry between bovine serum albumin and pancreatic autoantigen ICA6973, or to the effect of casein-immunostimulating hexapeptide.

Another theory is a relationship and/or overdose of the chemical nitrosamine. Nitrosamine is a known carcinogen. Scientists fear it may be possible to find nitrosamine within the body by ingestion of nitrites used in meats, foods and milk as a preservative. Sodium nitrite, used to prohibit the bacterium that produces botulinum toxin, and to keep the red color in meat, under the influence of acid (stomach) and/or heat (cooking) reacts with amines.

N-nitrosodimethylamine is present in cigarette smoke.

**Free Radicals**

Acute chronic enteroviral infection of peninsular tissue leads to b-cell destruction from abundance of free radicals, and in fact, in newly-developed diabetic, antioxidants are seriously absent, particularly Vitamins, C, B and K.

Free radicals fall under a broad category of reactions that involve homolysis of covalent bonds with the production of intermediates possessing unpaired electrons called free radicals. Heat/irradiation w/light must be supplied. Free radicals react by a chain reaction mechanism, in which the product of one step is reactant in the next step. Chain reactions, the most known of Oxygen, have three steps: 1. Initiation--radicals are made. 2. Propagation--radicals make new radicals. 3. Termination--all radicals die.

I believe the free radical approach is important when anemia is present in both the potential diabetic and the pregnant mother.
“Some proteins, called conjugated proteins, contain as part of their structure a nonprotein group called a prosthetic group. An example is the oxygen-carrying protein, hemoglobin. Each of the four polypeptide chains of hemoglobin is bound to a prosthetic group called heme. The four polypeptide chains of hemoglobin are wound in such a way as to give hemoglobin roughly spherical shape. Moreover, each heme group lies in a crevice with the hydrophobic vinyl groups of its porphyrin structure surrounded by hydrophobic side chains of amine residues. . . . The iron of the heme group is in the 2+ ferrous oxidation state and it forms a coordinate bond to a nitrogen of the imidazole group of histidine of the polypeptide chain. This leaves one valence of the ferrous ion free to combine with oxygen. . . . The fact that the ferrous ion of the heme group combines with oxygen is not particularly remarkable; many similar compounds do the same thing. What is remarkable about hemoglobin is that when the heme combines with oxygen the ferrous ion does not become readily oxidized to the ferric state. Studies with model heme compounds in water, for example, show that they undergo a rapid combination with oxygen but they also undergo a rapid oxidation of the iron Fe 2+ to Fe 3+. When these same compounds are embedded in the hydrophobic environment of a polystyrene resin, however, the iron is easily oxygenated and deoxygenated and this occurs with no change in oxidation state of iron. In this respect, it is especially interesting to note that X-rays studies of hemoglobin have revealed that the polypeptide chains provide each heme group with a similar hydrophobic environment.”

I believe in an anemic situation, the heme group no longer binds with Oxygen leaving Oxygen as a free radical where antioxidants are used up. A free radical-Oxygen is a highly reactive chemical.

Prevention

None of the following examples have been proven in human studies, only NOD mice (mice breed as diabetes prone mice). Some of the examples are noted because of cultural groups where diabetes has either increased or decreased 12, 13, 14.

Islet cell transplantation with Tuberculosis vaccinations

Much of the research is looking at simple islet cell transplantation where the Islets of Langerhorns that hold the beta cells in the pancreas are replaced. However, it would seem that if the reason for diabetes 1 was because of a virus attack, the immune system simply re-attacks with the new cells, even with auto immune drugs.

However, as discussed above, it seems that when the Swedish cultures withdrew vaccinations at birth, an increase of diabetes 1 resulted.

Alexander Rabinovitch, M.D., and colleagues at the University of Alberta in Edmonton knew that an environment free of pathogens increased the incidence of diabetes, but those infected with viruses had a decreased incidence of the disease.

Therefore, during transplantation of Islets of Langerhorns, they injected NOD mice with the dead tuberculosis bacteria which kept all the mice from developing Diabetes 1.
The “notion is that in autoimmune disease, if the immune system is busy fighting off microbial invaders, it doesn’t have time to attack its own cells.” Another theory is that “a given virus might have several strains, some that cause diabetes and some that don’t. Subsequently, being exposed to a particular strain of the virus that does not trigger diabetes might confer immunity to the other strains of the virus—and thus protection against diabetes.”

One known mechanism is that these injections increase the levels of interleukins, specifically cytokines, the cytokines which suppress destruction of the beta cells.

**Cytokines**

There are good and bad cytokines—these are cell messengers. Cytokines known as interleukin-2 and interferon gamma produced by the Th1 cells are seen during beta-cell destruction. Cytokines called interleukin-4 and interleukin-10 produced by the Th2 cells are seen when inflammation of the beta cells is reversed. Giving NOD mice the good cytokines during islet cell transplantation has shown to delay the recurrence of diabetes.

Cytokines are currently being manufactured for uses in diseases such as inflammatory bowel disease and septic shock, with clinical trials with humans in the near future.

**Diet and exercise**

Everyone knows that obesity, lack of exercise and poor diet will/can lead to adult onset diabetes simply because of a higher blood sugar and over-worked Beta-cells.

However, I am also concerned with diets that are loaded with food preservatives, cooked food which has denatured all proteins, and virtually no fruits or vegetables which are so important in providing antioxidants and fiber.

In a speech I did in December 1997, I sighted the Potgerger’s study where they feed cooked and uncooked food to over 900 cats for over 10 years. The heating of food denatured proteins—therefore cats were seriously protein deprived. Cats on raw food stayed healthy throughout the study, while those on cooked foods grew sickly and weak, and by the third generation all female cats were sterile.

The study by Benyshek and Martin, NOD mice who were protein deprived delivered pups that had a high rate of diabetes 11 is important work, I believe, in understanding both Diabetes I & II. In the milk, and nitrosamine, food preservative when combined with stomach acid or heat can make nitrosamine carcogenins and possibly destroy enzymes and other tissues.

**GAD Injections**

**Genetically engineered Gad plants**

This theory works like this: If a protein is attacked, then that protein would be helped by injecting the body with more of the same protein, like forming an army.
Researchers led by Dr. Anthony Jevnikar, in London, Ontario, London Health Sciences Centre have genetically engineered tobacco and potato plants to produce GAD. The plant then is used as a “oral vaccine” approach to reduce the immune responses to GAD. This vaccine interferes with the autoimmune response early by teaching the body to tolerate GAD. NOD mice when fed this vaccine, 10 out of 12 mice remained free from the disease, while the 12 of the control group, 8 developed diabetes.

My Theory:

Improving lifestyles, proper diet and exercise, providing enhanced protein rich pregnancy meals and breast feeding, would drastically lower the rate of diabetes. And it’s beyond time that we realize the consequences of lifestyle and diet. However, I believe we will prevent auto immune disease in the following manner.

When a child is born, gene therapists will decide if that child has a propensity for an auto-immune disease. A glitch is that several of the genes responsible for diabetes are also responsible for other auto-immune diseases and this might be solved in the near future, perhaps simply by looking at the family’s medical history. Next, I believe there we will see the advent of a new type of Immunization.

However, unlike immunizations which inject dead viruses that force the immune system to build an army, this immunization will supply an army. Similar to supplying Koscovo with an army, but before the attack, possibly at birth, or even in uterus.

This immunization for diabetes will most likely be a GAD protein complimented by Cytokines—the cell messenger. In other immune diseases, researcher, George S. Eisenbarth are successfully supplying NOD mice with the very protein that is destroyed before it is destroyed, like building a strong fence. Pilot studies in man and experimental studies of animal models have been completed of oral antigen therapy for multiple sclerosis with myelin basic protein, rheumatoid arthritis with collagen, and uveitis with retinal S protein. Therapy with insulin not only prevents development of diabetes of BB rats and NOD mice, but also prevents lymphocytic infiltrates into the islets and beta cell destruction. In humans, we have completed a small pilot trial of insulin therapy in high-risk relatives of patients with type one diabetes. Studies from Japan indicate that throxine therapy of patients whose Graves’ disease is in remission either during pregnancy or following methimazole treatment are protected from disease recurrence.

I believe this new immunization, an immunization of reinforcements before the war will be the answer for prevention, if not for cure.


Loratadine (Claratin): a brief summary of allergic rhinitis and the chemical nature of loratadine.

CHM 236

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April 23, 1999
Abstract

Loratadine is potent long lasting antihistamine that has no effect on the central nervous system. The compound is a tricyclic pyridine that works as a H-1 receptor antagonist. Broken down in the liver, the drug's active metabolite is descarboethoxyloratadine. There are no side effects associated with loratadine.

For thousands of people, the onset of spring means the beginning of a long ordeal with seasonal allergies. The most common form of allergies is hay fever or allergic rhinitis. This condition is brought on by the production of seasonal airborne pollen. As soon as the pollen season starts, individuals with this condition begin to suffer from its symptoms immediately. Hay fever begins by mucus membrane irritation about the nose, eyes, mouth, and roof of the mouth (1). Soon watery eyes, sneezing, and a clear watery discharge from the nose usually occurs. Some people develop headaches, coughing, and wheezing. The overall condition is usually associated with a prolonged bout in which the individual may even experience depression.

The most effective treatment for allergic rhinitis are antihistamines. These agents do not block the release of histamine, but rather they block the receptors on the surfaces of cells sensitive to histamine. While antihistamines are an effective means of treating hay fever, they are unfortunately associated with severe side effects. The most common and strongest side effect of antihistamines is drowsiness. In fact, antihistamines have such as strong sedative effect, they often cannot be used during the day. Therefore, many people have to suffer from their allergies in order to be productive during the day.

Researchers in search for a non-sedative antihistamine have developed loratadine. Sold as a prescription drug under the trademark name of Claritin, loratadine has both a strong antihistamine potency and lack of central nervous system interaction (2). This agent can be taken any time of the day and can be expected to have the same results as sedative antihistamines. Thus, loratadine is a 24-hour relief of seasonal allergies.

Loratadine is a tricyclic pyridine that is broken down in the liver into it's metabolite descarboethoxyloratadine. These two compounds are displayed in figure 1.

![Loratadine and Descarboethoxyloratadine](image)

Figure 1. Chemical structure of loratadine, and its major metabolite descarboethoxyloratadine.
Loratadine is a derivative of the antihistamine azadine, however in the synthesis of the two agents are commercially done by different mechanisms. The compound is usually prescribed in 10mg doses in tablet form, however Claritin is also available in a syrup form as well.

There are no adverse side effects associated with loratadine. Studies have shown the drug to have a preference only for peripheral tissues, thus avoiding the central nervous system. Patients with liver disease, tend to be less efficient at metabolizing the compound, but nonetheless, these individuals still seem to benefit from the medication. The following discussion in a review of the characteristics of loratadine and allergic rhinitis.

The area of the human body concerned with allergic reactions is the immune system. An allergen can be defined as any harmless substance that induces an inappropriate antibody response or hypersensitivity (3). For the purpose of this discussion, only allergic reactions causing allergic rhinitis (hay fever) will be reviewed.

When an allergen such as plant pollen enters the body of individuals who suffer from seasonal allergies, the immune system initially responds in the same manner as if a bacteria were introduced. The components of the immune system react with one another to form antibodies. But in the case of allergens, something very different happens. Instead of producing the typical antibodies during an infection, the immune system releases antibodies known as immunoglobulin-E (Ig-E).

These antibodies are unique in that they are primarily released only during an allergic response. Normally, when an antibody response is induced antibodies are created and released. These antibodies are designed to react with a certain chemical combination found of the foreign substance. This combination is known as an antigen. The antibody and antigen bind together through noncovalent forces. This is shown in figure 2.

![Figure 2. The interactions between an antibody and an antigen. This interaction depends on four types of noncovalent forces: hydrogen bonding, ionic bonding, hydrophobic interactions, and van der Waals forces.](image-url)
A major distinction between a hypersensitive (allergic) and a humoral (normal antibody) response is that the antibodies created in the hypersensitivity response do not initially bind to the foreign antigen. Instead, the Ig-E antibody binds to a mast cell first. This binding occurs at the tail end portion of the antibody (Fc portion). This is shown in figure 3 and 4. In contrast, during a normal humoral response, the Fab portion of the antibody binds to the antigen first, leaving the Fc portion exposed.

![Diagram of Ig-E antibody structure](image)

**Figure 3.** Prototypical structure of an immunoglobulin such as Ig-E. The two pairs of Fab chains represents the antibody-antigen binding portion. The Fc part of the structure binds with the body's own structures. In the case of Ig-E, the Fc portion binds to Fc receptors on mast cells.

![Simplified mast cell with Ig-E antibodies attached by their Fc portion](image)

**Figure 4.** Simplified mast cell with Ig-E antibodies attached by their Fc portion. At this point, the mast cell is said to be sensitized.

Most individuals create and secrete Ig-E in response to parasitic infections. Atopic individuals however, have a genetic predisposition to release Ig-E in response to an allergen. These people not only produce higher concentrations of Ig-E, they also have more Fc receptors on their mast cells compared to normal individuals. The prevalence of allergies has a strong genetic link. In families in which one parent has an allergy, allergies develop in about 40% of the offspring (4). If both parents have atopic disease, the incidence in offspring rises to 80%.
As is shown in figure 5, the allergic reaction begins when an allergen is cross-linked with two separate Ig-E molecules. This cross-linkage triggers a cascade of biochemical reactions that leads to the degranulation of the mast cell.

![Diagram of mast cell and allergen interaction](image)

Figure 5. The B cell (white blood cell) produces Ig-E antibodies. The Ig-E binds to the Fc receptors on the mast cell's cellular membrane. Further exposure to the same allergen causes linkage with the Fab portion of the Ig-E antibody. Cross-linkage results in the degranulation of the mast cell.

The granules released by the mast cells are pharmacologically active mediators. These mediators travel throughout the blood stream and effect a variety of cells. The biological effects induced by these mediators include vasodilatation and smooth-muscle contraction. The major mediator and the one primarily responsible for allergic rhinitis is histamine. Figure 6 describes the synthesis of histamine.

![Diagram of histamine synthesis](image)

Figure 6. Histamine is an amine found throughout the body. The amine is formed by the decarboxylation of L-histidine. The reaction is catalyzed by histidine decarboxylase (5).
Certain receptors on target cells in the host tissue are very receptive to histamine. Binding of histamine to the H-1 receptors initiates contraction of bronchial smooth muscle cells. This leads to bronchial constriction. Histamine-H-1 receptor binding also induces vascular permeability which leads to edema. These two actions typically result in the clinical manifestations of seasonal allergies, most notably rhinitis (inflammation of membranes lining the nose). Reactions to allergens often occur rapidly in sensitized individuals. This is because both mast cells and cells sensitive to histamine are located on the peripheral tissues such as mucus membranes in the nose and bronchial tissues in the lungs.

Antihistamines are most often used to combat the symptoms of allergic rhinitis. The term, antihistamines usually refers to drugs that block histamine from binding to the H-1 receptors on cells. These drugs do not actually block the release of histamine, Ig-E production, or allergen-antibody interactions. All antihistamines have similar effects, but usually differ in their undesired side effects. Some antihistamines have anticholinergic effects. Anticholinergics block acetylcholine, an important neurotransmitter. This can cause confusion and blurred vision. The elderly are sensitive to anticholinergics because with age, the amount of acetylcholine decreases. A second and more commonly experienced side effect is drowsiness. Antihistamines are well-known for their sedative effects. In fact, many over-the-counter sleep aids contain antihistamines.

Because of the potent sedative nature of antihistamines, researchers have investigated possible non-sedative agents. Antihistamines naturally have sedative effects due to their attraction to H-1 receptors. When antihistamines enter the central nervous system, they act the same as they would in other parts of the body. In other words, they compete with the body's own mediators (including neurotransmitters) for H-1 receptors. By blocking the H-1 receptors of nerve cells, antihistamines slow down the mechanisms of the central nervous system, which leads to drowsiness.

Nearly all of the known antihistamines have sedative effects. The drowsiness caused by these drugs inhibits their use. Patients are usually restricted to using these agents when the drug's sedative effects do not hinder daily activities. Therefore, people usually suffer from allergic rhinitis most of the day. Scientists have found three antihistamines that act as H-1 blockers and do not cause sedative effects. These include terfenadine, astemizole, and loratadine. While all three agents have shown to be effective, loratadine had displayed the greatest antihistamine potency.

Loratadine had quickly become the agent of choice for the treatment of seasonal allergies. Sold under the trademark name of Claratine, loratadine is the 19th best selling prescription drug on the market (6). The popularity of this drug has developed due to its unique combination of potency, long lasting effects, and selective peripheral H-1 receptor blocking activity. Because loratadine acts on peripheral tissues, there are no adverse reactions involving the central
nervous system. The diverse properties of loratadine has enabled it to be the first real form of a 24-hour relief for seasonal allergies.

Loratadine is a white powder with a molecular weight of 382.89 and empirical formula of C_{22}H_{23}ClN_{2}O_{2}. Due to its nonpolar nature, loratadine is not soluble in water, but is readily dissolved in acetone, alcohol, and chloroform. The formal chemical name for the compound is ethyl4-(8-chloro-5,6-dihydro-11H-benzo[5,6]cyclohetal[1,2-b]pyridin-11-ylidene)-1-piperidinecarboxylate. The structural formula for loratadine is diagrammed in figure 7.

![Structural formula of loratadine](image)

Figure 7. Structural formula of loratadine.

While loratadine is a unique antihistamine, it is a derivative of azatadine (7). Azatadine is a compound that has limited central nervous system side effects, however, the agent is a poor antihistamine. The structural formula of azatadine is shown in figure 8.

![Structures of loratadine and azatadine](image)

Figure 8. Structures of both loratadine and azatadine.

Although loratadine is a derivative of azatadine, the presence of chlorine in the phenyl ring causes some problems during the synthesis of the compound. In the synthesis of azatadine, two reduction steps are required (8). This cannot be done with loratadine intermediates. This is because the chlorine atom gets removed during the process. A second complication in the synthesis of loratadine is in the formation of the tricyclic ring. Forming this structure with polyphosphoric acid, results in isomers chlorinated in the 8 or 10 position.
Early attempts to overcome this problem were done by an inefficient alkylation and reduction process. This resulted in yields of less than 35%. The products are then acylated through Friedel-Crafts and the final product was obtained through a Grignard reaction. This entire process resulted in loratadine yields of less than 5%.

This extremely inefficient form of synthesis was not acceptable for pharmaceutical purposes. A more economical pathway of synthesis was discovered and used by Schering-Plough, the makers of Claritin. Figure 9 diagrams the synthesis of loratadine.

![Chemical diagram](image)

Figure 9. Step-by-step diagram of the synthesis of loratadine.

The starting product 2-cyano-3-methyl-pyridine (3), is first treated with H$_2$SO$_4$ and t-butyl alcohol (a). The t-butyl group was added in order to prevent the self-condensation of the nitrile. The t-butylamide (4) is then alkylated with m-chlorobenzyl chloride (b). This gives rise to pyridine (5) at yields approaching 92%. A small quantity (<10%) of dialkylated by product (6) is also made. The amide (5) is then converted to a nitrile (7) by using phosphorusoxochloride (c). The next step requires the Grignard reagent N-methylpiperdyl magnesium chloride (d). This reagent gives rise to the imine (8). Hydrolysis of the imine using hydrochloric acid results in the keto hydra chloride (9) (e). This product is then isolated and converted to loratadine.
Loratadine is first created by the addition of hydrogen fluoride in boron trifluoride, to the keto hydorchloride (9). The reaction results in the product in figure 10.

![Structural formula of the last intermediate in the synthesis of loratadine.]

Figure 10. Structural formula of the last intermediate in the synthesis of loratadine.

This compound is then converted to loratadine by reaction it with ethyl chloroformate in toluene. The overall yield of loratadine for this method is 57%.

Loratadine is rapidly absorbed following the oral absorption of 10mg tablets. The onset of action occurs within 1-3 hours. The duration of drug action is greater than 24 hours. Loratadine has a high first pass effect and is almost completely metabolized in the liver. The major active metabolite of the compound is descarboethoxyloratadine (9). Figure 11 shows the synthesis of loratadine to the hydroxylated derivatives that are excreted from the body.

![Abbreviated diagram of the metabolism of loratadine.]

Figure 11. Abbreviated diagram of the metabolism of loratadine.

Loratadine is first hydrolyzed to the piperidinylidene amine 2 (also known as descarboethoxyloratadine). This amine is then hydroxylated at several positions. These hydroxylated derivatives may be conjugated and are found to be ultimately excreted in the urine in their free or conjugated form. These hydroxylated compounds (3a and 3b), are found to each exist as a pair that equilibrate at room temperature. This effect is most likely attributed to the existence of conformational diastereomers. The two metabolites exist as a
mixture of two spectroscopically similar components which can be readily separated at -25 degrees C.

Currently there are no known serious side effects associated with loratadine. The only individuals found to have difficulty in using loratadine are persons with liver disease. Because loratadine is metabolized nearly exclusively in the liver, liver impairment makes effective delivery of the pharmaceutical agent difficult. Patients with normal liver function have peak plasma concentrations of loratadine of roughly 50%. Subjects with chronic alcoholic liver disease achieve peak serum concentrations which are nearly double those observed in patients with normal livers. So individuals who have health livers are able to metabolize loratadine twice as effectively as those who have impaired livers.

Loratadine has shown to offer tremendous relief for people that suffer from allergic rhinitis. The compound has shown to be as strong if not stronger than most of the available antihistamines. More importantly, loratadine has no sedative or anticholinergic effects. Instead of dreading the flowers of spring, everyone can now enjoy changing seasons with a clear head.
Works Cited


