Case Handout

for

"A Diet to Die For: An Exploration of Oxidative Phosphorylation"

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> **"By the end of the two and a half decades between 1940 and 1965, the field of oxidative phosphorylation was littered with the smouldering conceptual remains of numerous exploded energy-rich chemical intermediates; the remarkable uncoupling action of 2,4-dinitrophenate [dinitrophenol] … remained obscure; and the process of hypothesis-building, needed to keep faith with the chemical-coupling notion, reached such fantastic proportions as to be hardly intelligible to those outside the field."**
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> ***—Peter Mitchell (1978 Nobel address)***

Prologue: **Coal-tar dyes were used in the late 19th century to color food; one favorite in Europe was Martius yellow (dinitronaphthol), used in pastries and macaroni where the color implied the foods were rich in eggs. A related dye with similar properties was Victoria Yellow (dinitocresol). Some deaths occurred due to the use of these dyes, which called attention to their potential danger in food products. This led French and German scientists to study toxicity of these coal-tar dyes with dogs: in moderate doses, the symptoms included increased respiration and rise in body temperature (to 44° C in one case) before death ensued. Because of their toxicity, these two dyes were banned in Europe for use in coloring food by 1900, and within a few years were similarly banned in the United States, although seven other coal-tar dyes could legally continue to be used in food coloration.**

During and after World War I, munitions factories in France commonly made explosives from 40% 2,4-dinitrophenol (DNP) and 60% picric acid (trinitrophenol) rather than the trinitro-toluene (TNT) manufactured in Britain and the U.S. Overweight workers in these factories were observed to lose weight, felt fatigued, and sometimes displayed excessive sweating and elevated body temperatures. Various studies between 1910 and 1920 confirmed in animal experiments the previously observed toxicity of dinitrophenol, without being able to identify the mechanism of its action. One interesting possibility, that increased heat production was due to "a stimulation of cellular oxidation," became favored—a remarkable insight, considering that ATP itself was not discovered until 1929!

In the U.S., Stanford scientists learned of the early French studies and repeated many of those experiments with similar outcomes. This appeared to present a genuine method for "free" weight loss, and by 1933 physicians had carried out clinical studies, establishing dosage levels that were able to hold the metabolic rate of obese patients at a level 40% higher than normal with no adverse effects. This resulted in weight loss without dieting, which was great news for patent medicine promoters, who seized upon this finding with glee. They began marketing "miracle cures" for obesity, and some physicians started to prescribe DNP as a weight loss remedy. By 1935, more than 100,000 people were estimated to have been "treated" with DNP. Unfortunately, deaths attributable to DNP had begun to be reported. Concern spread after a 1933 *Newsweek* headline blared "Diet and Die with Excess Alpha Dinitrophenol," which told "of a physician who had been 'literally cooked to death' when he took an overdose of the drug in an attempt to lose weight rapidly" (Parascandola, 1974).

The amount of dinitrophenol that causes harmful effects varies among individuals, because the amount necessary to realize moderate weight loss (therapeutic dose) and the amount that produces death (lethal dose) are separated by less than a factor of ten. Given the propensity of patients to believe that "if one pill is good, two pills must be twice as good," it is easy to see how DNP was able to claim so many lives in so short a time. Common signs and symptoms include increased basal metabolic rate (the rate that you use energy at rest), rapid breathing, increased sweating, a feeling

of warmth (even in chilly or cold conditions), and weight loss. Your heart rate, breathing rate, and body temperature rise. Cataracts, skin rashes, and decreases in white blood cell counts are also associated with DNP ingestion.

At that time, the government had no control over "cosmetic" medications if no medical claims were made on the label—and in any case "obesity" was not considered a disease. To a large extent, the deaths caused by misuse of DNP forced the adoption of a new stricter "Food, Drug and Cosmetic Act" by Congress in 1938. Shortly thereafter, DNP was banned as a weight loss drug by a newly formed governmental agency: the U.S. Food and Drug Administration (FDA). This was one of its first official actions, and in fact the abuse of this "drug" may have been instrumental in Congressional chartering of the FDA, an institution that we now take for granted. The current mandate of the FDA has become to monitor for safety and efficacy "all food except for meat and poultry; all prescription and non-prescription drugs; all blood products, vaccines, and tissues for transplantation; all medical equipment and all devices that emit radiation, including microwave ovens; all animal drugs and feed, and even all cosmetics." Yet even today, DNP is available over the Internet.

It took another two to three decades to confirm that 2,4-dinitrophenol acted as an uncoupling agent in mitochondria both *in vitro* and *in vivo*, though the mechanism of its action remained obscure until the late 1960's. Due to the pioneering efforts of Dr. Peter Mitchell, supported by those of his scientific colleagues open-minded enough to consider his "Chemiosmotic Hypothesis" as a serious alternative to the "Chemical Coupling Hypothesis" (previously assumed by many to be correct), we now understand how DNP works. In the class period(s) ahead you will explore the mechanism of DNP action to illuminate the currently accepted cellular model for coupling between the electron transport chain and ATP synthesis in the process called "oxidative phosphorylation."

Background reading for class: The text chapter covering oxidative phosphorylation.

Ask yourself: 1. What is meant by "coupling" of oxidative phosphorylation?

2. What is meant by "uncoupling" of oxidative phosphorylation?

3. Under what kinds of conditions will these effects occur?