DR. WATKINS: Our next speaker is Adrian Reuben from the Medical University of South Carolina in Charleston.

DR. REUBEN: Thank you. I was obviously very flattered when John Senior asked me to give this talk and it made sense to explain Hy's Law, but then having sat here for the past three hours and had Hy's Law explained to me, I'm a little uncertain as to my role except perhaps for some reality testing.

I have to put in context Hy's contribution and remind you that toxicity has been with us for thousands of years. People have been treating themselves and poisoning each other or poisoning themselves and treating each other, as far back as recorded history.
Drug Hepatotoxicity: A History

Toxins and Poisons of the Ancient World

- 1774 Bang: Arsenic hepatotoxicity
- 1849 Chloroform-induced hepatic necrosis
- 1888 Phosphorus and arsenic: liver/kidney necrosis
- 1923 1st Idiosyncratic drug reaction - cinchophen for gout
- 1940 Arsphenamine-induced cholestasis
- 1940s Hepatitis-like drug reactions, eg aminothiazole
- 1940s-1970s isolated case reports and small series

Probably the first example of hepatotoxicity was in the late 18th Century with arsenic, which was a favorite not for killing people but for treating syphilis. Then chloroform came along, causing hepatic necrosis although it actually took 50 years to recognize that that had happened. Phosphorus and arsenic were still around in the late 19th Century but caused liver and kidney toxicity, and possibly the first idiosyncratic reaction or drug reaction was for gout treatment. Arsphenamine, of course, the cure for syphilis was found to cause cholestasis, and the first really hepatitis-like reactions were again only described in the 1940's. Interestingly, Himsworth, when he gave his famous Lowell lectures in Boston in 1947, made a great point of describing a case of an aminothiazole hepatotoxicity, as it seemed to be unrecognized.

But between the 1940s and 1970s, there were really only isolated case reports and small series and the field really was very dysfunctional and uncoordinated until 1978.
This is Hy Zimmerman's first textbook, which was published then. The second edition in 1999 was published just a few weeks after Hy's death, but I'm told that he actually saw the cover of the book and, of course knew all about it; it still is a great standby. It increased in size by about 30 percent and I think it's about 50 percent heavier if you lift it up.
Hy Zimmerman (1914-1999)

- Intrinsic vs idiosyncratic hepatotoxicity
- Hepatitic vs cholestatic reactions
- Cholestasis: hepatocellular, hepatocanalicular, ductular

**Drug-induced hepatocellular jaundice is serious**
Later referred to as Hy’s Rule or Hy’s Law

So who was Hy Zimmerman? Many of the, I was going to say older members, I mean longstanding members of this group, knew Hy very well. He was born in Rochester, New York, in 1914, where he was raised and went to school but by the restrictions that existed then in many universities, called *numerus clausus*, he was not allowed to go to medical school in Rochester because they had too many Jews. So he was forced to go to Stanford. What a terrible banishment, (laughter), where he graduated. But the war came along and he actually finished his medical training in the Army where he was very well trained in tropical medicine. I see there are some military people around, however with inimitable military wisdom, he was then sent to northern France where he was serving near the Battle of the Bulge. While his senior medical officers were doing serious investigation on the neuropsychiatric effects of alcohol, mostly on themselves, Hy was then able to polish his clinical skills and wrote a number of papers, one on hepatitis, and then later his first on -- hepatotoxicity.

After military service, he spent some 50 years in the VA system, was a most distinguished individual, chaired various departments of medicine, was known not only for his teaching but also for his great humanity and everybody who knew Hy, knew him as just a wonderful, honest and warm human being.

His contributions really were considerable. He, if he did not discover, at least made clear the difference between intrinsic and idiosyncratic hepatotoxicity. He distinguished between hepatitic and cholestatic reactions. He defined cholestatic reactions on an anatomical basis, namely whether it was hepatocellular (which is what we’re talking about here today) or hepatocanalicular or ductular and then made this seminal induction that drug-induced hepatocellular jaundice is a serious entity.

Now this was later referred to as Hy’s Rule or Hy’s Law but I would argue that it should be Hy’s Hypothesis.
Estimated Case Fatality Rates (%) in Drug-Induced Acute Hepatocellular Injury

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Cinchophen</td>
<td>50</td>
<td>Cinchophen</td>
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<tr>
<td>Dantrolene</td>
<td>10</td>
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<tr>
<td>Halothane (14-70%)</td>
<td>50</td>
<td>Halothane</td>
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<tr>
<td>Iproniazid</td>
<td>15</td>
<td>Iproniazid</td>
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<tr>
<td>Phenytoin</td>
<td>&gt;40</td>
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<td>Isoniazid</td>
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<td>Isoniazid</td>
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<tr>
<td>&lt;Methyl dopa</td>
<td>10</td>
<td>&lt;Methyl dopa</td>
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<td>Ticrynafen</td>
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*Hy's observations:* Drugs are a relatively rare cause of hepatocellular injury but when jaundice occurs it is a grave event.

*Hy did not mention specifically height of bilirubin or aminotransferases (AT). Jaundice implies bilirubin ≥3. Hepatocellular injury αAT 2x-200x, now defined by αAT/αAlk phos ≥5x*

This is based on -- these case reports, as it were -- that he illustrated in the first and second edition of his book. Why phenytoin disappeared I do not know because it's a significant hepatotoxin, but you can see that the estimated case fatality rates were as low as around about 10 percent with -- we've lost the alpha symbol -- alpha methyl dopa, but as high as 50 percent with other drugs. Here's Halothane, 14 to 70 percent fatality, and so Hy's observations that though drugs are a relatively rare cause of hepatocellular injury, when jaundice occurs, it is a grave event as he described it.

Now Hy didn't mention specifically, the height of the bilirubin or the height of the aminotransferases. This is where the reality testing comes in. Now jaundice implies that the bilirubin is in excess of three because -- the symbols on the slide have changed, but at any rate, it means in excess of three, because that's the level at which most clinicians ought to be able to detect jaundice in a patient's eyes in good light and with a good wind behind them.

Hepatocellular injury in these cases or these reports mostly had aminotransferases in the 5 to 15 or 50 or even 200-fold elevated. There were a few spotty necrosis cases where they were only twofold elevated but they were mostly substantially higher.

Now in the DILI network, hepatocellular injury is defined as a ratio of aminotransferase elevation compared to alkaline phosphatase elevation, in excess of five. So that's how it's defined in the DILI network.
Drug-Induced Liver Injury (DILI)

Hepatitis: adults—overall 10% due to DILI, age over 50 yrs – 40%

France: 14/100,000 or 8,000/yr, 12% hospitalized, 500 deaths, under-reported - Sgro 2000
Switzerland: Hospitalized patients: Incidence 1.4% - Meier 2005
Sweden: Severe DILI: Death/transplant rate – overall 9%, - hepatocellular injury 13% - Bjornsson 2005
Spain: DILI with jaundice: death/transplantation 12% - Andrade 2005

Overall, it appears that in adults, about 10 percent of all hepatitis cases might be due to DILI, but when you get to age over 50 or more, it's up to about 40 percent. And in reports from France and Switzerland -- here you see an excellent report from Sgro back in 2000, where they summise that the reporting of DILI is really underestimated and in a very tight population, what they found would predict about 8,000 cases per year in France, 12 percent who were hospitalized resulting in 500 deaths. Peter Meier looked at hospitalized patients in Switzerland and DILI accounted for 1.4 percent of all patients in the hospital.

And then there are two very nice reports, one from Sweden and Spain, I'll go into a little in detail in a moment, that the death rate or transplant rate for severe DILI or DILI with jaundice in the Spanish report is around about 10 to 12 percent, somewhat consistent with the lower limit of Hy's Hypothesis. And just to remind you that in the United States, 6 percent of acute liver failure patients who come to transplant are thought to be due to DILI.
DILI In the Acute Liver Failure* Study

*Encephalopathy and coagulopathy (INR>1.5)

n=116

- **Antimicrobials (43%)**
  - **Antituberculous drugs (19)**
    - INH Alone (9)
    - INH with rifampin +/- pyrazinamide +/- ethambutol (7)
    - Rifampin +/- pyrazinamide without INH (3)
  - **Sulfur drugs (10)**
    - Co-trimoxazole (6), sulfadiazine (1), sulfasalazine (3)
  - **Other antimicrobials**
    - Nitrofurantoin (7), doxycycline (3), ceftriaxone (1)
    - Ciprofloxacin, amoxicillin-clavulanic acid, clarithromycin – 1 each
    - Antifungal – ketoconazole or itraconazole (3), terbenafine (2)
    - Anti-retroviral – abacavir, didanosine - 1 each

Now in the Acute Liver Failure Study Group that the NIH funds consisting of about 23 sites, of which Will Lee is the principal investigator, these patients would be included if they had encephalopathy and coagulopathy. So we're talking about progressive severe disease. There are around 130 that I have now, but these are the analyses I have done of 116: 40 percent used antimicrobials, the majority of which have been antituberculous drugs and the majority of those are INH and a few percent with non-INH drugs. Sulfa drugs are a common cause but a host of other antimicrobials of which nitrofurantoin is the most frequent and you see lots of others there, for example, amoxicillin-clavulanic acid, antifungal agents and some antiviral agents.
The next group are sort of neuro-active drugs: antiepileptics, phenytoin being the most popular, including disulfiram, that's the second most important, but it's not strictly neurotoxin. Endocrine drugs as you see, troglitazone (Rezulin) and the ones with the asterisks, the ones that have subsequently been withdrawn. The same too with statins; the only two that convincingly caused severe DILI were cerivastatin which as you know was withdrawn from the market because of rhabdomyolysis, not because of hepatotoxicity.
DILI In the Acute Liver Failure Study

• **Vascular drugs (3%)**
  – Methyl dopa (2), hydralazine (1), cocaine (1)

• **Herbals, dietary supplements etc (9%)**
  – Usnic acid, Ma Huang, Kava-kava, Horny Goatweed, and others often unspecified or unrecognizable (11 cases)

• **Oncologic Drugs and Biologicals (4%)**
  – BCG (1), interferon beta (1), melphalan (1), gemtuzumab (1), zafirlukast (1)

And then finally we have drugs which are vasoactive. Herbal and dietary supplements account for nine percent and very often one can't tell which herb or supplement they were actually taking. They were very often mixtures, and there's an increasingly inclusion of onocologic and biological drugs. We had about five or six, five of those in the group that I analyzed. So the top seven results for DILI that cause acute liver failure are shown here and the, no surprise, spontaneous survival rate is from 0 to about 50 percent. And, if you look at those who died or required liver transplantation, it's anywhere between 50 and 100 percent. So this is serious stuff.
But it's important that observations like these be made. Unlike clinical trials where you try to control concomitant drugs and know what they are, in this data set, over 75 percent of the drugs were either taken in combination with other drugs or alcohol. So clinical trial experience may not reflect routine use, and I think it's extremely important. The second is the duration of exposure that was usually less than about four months, maybe as short as a few days or as long as half a year, and for nitrofurantoin, exposure was greater than a year for all but one case. So one has to be careful. The signal may not actually be detected if the clinical trial is relatively short.
DILI In the Acute Liver Failure Study

- Over 75% of drugs taken with other drugs, alcohol etc
  - Clinical trial experience may not reflect routine use
- Duration of exposure, where reported, was usually less than 4 months but could be as short as a few days, or as long as 150-180 days. For nitrofurantoin, exposure was greater than 1 year for all but 1 case
  - Signal may not be detected in short clinical trials
- 11 cases involved drugs that were later withdrawn by FDA:
  - bromfenac (4), troglitazone (4), cerivastatin (2), nefazadone (1)
  - Most drugs that caused ALF are still in clinical use
  - Other Hy’s Law positive drugs still in use: phenybutazone, labetalol, diclofenac, sulindac
  - What is risk: benefit threshold?

And the third point that I want to make is that 11 cases involved drugs that were later withdrawn by the FDA, as shown here. But the important point is that most drugs that cause acute liver failure are still in clinical use and some of them are known to be Hy's Law positive in other series such as phenybutazone, labetalol, diclofenac and so on. And so the whole point about whether a Hy's Law case leads to the removal of the drug -- as might have been done for isoniazid -- or the continuation drug, depends on a lot of other things, either benefit, risk-benefit threshold which is something you can't decide in the clinical trial. That has to be a practical consideration.
Overall Outcomes of DILI ALF

• Spontaneous survival 30/116 or 26%

• Transplanted 45/116 or 39%
  43/45 transplants survived i.e. 96%
  **Overall survival 63%**

• Died 41/116 or 35%,
  **Dead or transplanted 74%**

Spontaneous survival in these cases was extremely low. Those who were transplanted did very well. Actually 96 percent survived but if you look at those who died or required transplantation, it was three-quarters. So if a Hy's Law type of case actually gets to acute liver failure, the outlook is extremely poor.
In Practice, What is Hy’s Law?

- Based on Hy Zimmerman’s inductive reasoning, term coined by Robert Temple in 1980s as a “biomarker” of drug hepatotoxicity, a signal for potential serious risk. Applied only to hepatocellular toxicity, not to cholestatic reactions or other liver diseases. *Adapted* from Hy’s conclusions.

- **Hepatocellular injury**
  - Elevated ATs, but at what level?
    - 2x Upper Limit of Normal (ULN), but too common and benign
    - 3x ULN
    - 8x ULN, 10x or greater ULN; what is signal: noise threshold?

- **Plus Jaundice** – implies injury that impairs bilirubin excretion, for which there is high capacity before accumulation occurs.
  - Bilirubin threshold 3 mg/dL for seeing jaundice but imprecise
  - Bilirubin $\approx$ 2 mg/dL, 2x ULN, still implies impaired liver function

So what is Hy's Law? Now Hy Zimmerman did inductive reasoning. He had a huge number of observations of his own many cases from the literature, cases he was consulted on, and Robert Temple, as you've been told, coined this term as a biomarker of drug hepatotoxicity or a signal for potential serious risk.

It's important to remember this applies only to hepatocellular toxicity not the cholestatic reaction. So we need something for that or indeed to other liver diseases. Hy’s law was derived from Hy's conclusions, it was not verbatim from Hy's writings.

So what level of aminotransferase elevation are we going to look for. Two times the upper limit of normal is clearly trivial because it's extremely common and usually benign. Bob Temple took 3 times the upper limit of normal but as you know, there have been proposals for 5, or 8, or 10 times, and so what is the signal to noise threshold at which you make that decision, and that's really not been totally resolved.

And they have to have jaundice. This implies that the injury, as somebody said just a moment ago, impairs bilirubin excretion for which the liver has high capacity before bilirubin accumulates in the circulation. It's a bit like renal function. You can draw some sort of exponential curve.

But the bilirubin threshold for seeing jaundice in the eyes would be three, but we know that's imprecise, and so the value of two which is twice the upper limit of normal was selected as being measurable but also implying significant deterioration if you're lacking in global liver function.
Defining Hy’s Law Cases

- **Hepatocellular injury**
  - Requires exclusion of other causes of liver injury: viral hepatitis, fatty liver, alcohol damage, ischemia, etc
  - Causality assessment
  - ALT alone may not indicate serious damage, but higher frequency and degree of ALT are also predictive of serious hepatotoxicity. “Rezulin Rule”, ALT>3x ULN in >2% subjects

  Troglitazone 1.9% vs placebo 0.6%
  Bromfenac 10% vs ibuprofen 1%, placebo 0%
  Ximalagatran 7.9% vs warfarin 1.2%

  Lewis JH; Pharmacopid Drug Saf 2006; 15:221-229

  - Need to distinguish transient mild ALT (tolerance) from toxicity
  - Can these rules be applied to patients with pre-existing liver disease?

Now in terms of hepatocellular injury, it's been said that we have to exclude other causes but this brings up a very important point and that's one of causality assessment which I don't believe is being discussed very much in this conference. It's not just the exclusion of other drugs and so forth. It's a whole range of assessments. Is the timing correct? Did the patient really take the drug? What else were they taking? Were there over-the-counter medications or remedies that you don't know about?

An elevated ALT alone doesn't necessarily indicate serious damage unless it occurs at high frequency, and a high degree of ALT elevation which may be predictive of serious hepatotoxicity without bilirubin elevation, for which James Lewis has proposed this concept of a Rezulin Rule. I suppose the alliteration is why he has RR. Maybe it's because he wants “Rezulin” as opposed to “troglitazone rule”. But at any rate, if the ALT is more than three times the upper limit of normal, in greater than two percent of subjects, this may be a signal for bad hepatotoxicity. And here's a group of drugs that were either not approved or were withdrawn, and if you look in the clinical trials, their prevalence of ALT greater than threefold range from 1.9 to 10 percent whereas the placebo or the control drug was 1 or 2 percent. We need to distinguish those people whom we have said already have this mild increase in ALT, so-called tolerance, from toxicity and the real question is, as will be discussed I think tomorrow, can these rules be applied to patients with preexisting liver disease who already have a baseline ALT elevation which may fluctuate and may even have a very slight bilirubin elevation, and there's a real problem of sorting that. We can't sort it out in so-called normal individuals. Sorting it out in diseased individuals is going to be very difficult.
Defining Hy’s Law Cases

• **Hepatocellular injury**
  – requires exclusion of other causes of liver injury: viral hepatitis, fatty liver, alcohol damage, ischemia, etc
  – causality assessment
  – ☯AT alone may not indicate serious damage, but higher frequency and degree of AT☯ are also predictive of serious hepatotoxicity ("Rezulin Rule", AT>3x ULN in >2% subjects)
  – need to distinguish transient mild ☯AT (tolerance) from toxicity
  – can these rules be applied to patients with pre-existing liver disease?

• **Jaundice**
  – must exclude other causes of cholestasis: extra- and intrahepatic (screen with elevated alkaline phosphatase)
  – severity of jaundice is an important predictor of mortality

We must exclude obviously other courses of cholestasis, and alkaline phosphatase is used to screen for that but, notwithstanding, if the jaundice is severe, it may be an important predictor of mortality in both cholestatic and hepatitic reactions. The higher the bilirubin, the worse the patients do overall. So this may be an important subset if you like.
Validation of Hy’s Law

- **Andrade et al. Gastroenterology 2005; 129: 512-521**
  1994-2004 Spanish Registry:
  461 DILI cases - defined by RUCAM causality
  liver transplant/death in hepatocellular injury:
  
  jaundiced (71%) 11.7% , non-jaundiced 3.8%
  drugs: anti-microbials 32%, CNS 17%, musculoskeletal 17%
  ALF 18 cases: 12 died, 6 liver transplant
  Risk factors for ALF: female gender, hepatocellular damage, bilirubin elevation

Now Hy's Law has been validated recently, a couple of years ago. Here's the data from the Spanish registry where they had 461 DILI cases diagnosed using the RUCAM method of causality. Liver transplant or death in hepatocellular injury was 11.7 percent in the jaundiced group that comprised 71 percent of the cases, but was only 3.8 percent in the non-jaundiced, and the major drugs were antimicrobials. In their experience it was amoxicillin-clavulanic acid and of the ALF cases, 12 died and 6 required liver transplantation. The risk factors in their series were female gender, hepatocellular damage and bilirubin elevation, in other words, what would fulfill Hy's Law.
Validation of Hy’s Law

• Andrade et al. Gastroenterology 2005; 129: 512-521

1994-2004 Spanish Registry:
461 DILI cases - defined by RUCAM causality
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ALF 18 cases: 12 died, 6 liver transplant
Risk factors for ALF: female gender, hepatocellular damage

• Bjöörnsson and Olsson. Hepatology 2005; 42: 481-489

1970-2004 Swedish Registry:
784 DILI cases – defined by RUCAM causality
liver transplant/death: hepatocellular injury 9.2%, cholestatic 7.8%, mixed 2.4%
drugs: antimicrobials (especially antituberculous), NSAIDs, anticonvulsants
Risk factors for death/transplantation:
   hepatocellular injury – AST and bilirubin, cholestasis/mixed injury - bilirubin

In a registry that extended over 34 years in Sweden, they had 784 DILI cases. Again RUCAM was used for causality, but here's an interesting point, hepatocellular injury resulted in death in 9, or transplant in 9.2 percent, again the lower limit of the Hy's Law observation, but cholestatic disease also resulted in death and for some reason the mixed picture, hepatocellular and cholestatic, were really much lower in outcome, and again drugs were the antimicrobials and especially, antituberculous drugs, NSAIDs and anticonvulsants, and again the risk factors for death or transplantation in hepatocellular injury were AST and bilirubin and in those with cholestasis it was bilirubin alone.
# Hy’s Law: Current Status

- **Concept is still valid:**
  - Drug induced hepatocellular jaundice carries ~10% mortality risk
- **Parameters for AT yet to be scientifically defined prospectively**
  - which “stopping criteria” should be used?
    - ALT > 3x ULN + bilirubin > 2x ULN (Hy’s Law)
    - ALT > 8x ULN
    - ALT > 5x ULN for 2 weeks
    - ALT > 3x ULN with systemic symptoms
- **Previously unappreciated fatality risk of drug-induced cholestasis**
- **Should hepatic panel monitoring be supported for drugs with greatest hepatotoxic potential in clinical practice?**
  - Antibiotics/antimicrobials, anticonvulsants, NSAIDs and other musculoskeletals
- **Continuing importance of causality assignment to “verify” that hepatotoxicity is truly drug-induced.**

So what is the current status of Hy's Law or Hy's Hypothesis? I think it's still valid. Drug-induced hepatocellular jaundice carries at least a 10 percent mortality risk. The parameters for choosing ALT elevation are yet to be defined prospectively, and which stopping criteria should be used, and as far as I understand it, the FDA has a range of both that are being considered, namely the standard Hy's Law, the eightfold elevation or the fivefold elevation that lasts for a couple of weeks or threefold elevation associated with some systemic symptom such as abdominal pain, fever, malaise, nausea, what have you.

The risk of fatality of cholestasis has previously not been appreciated and if you read Hy's book, he thought that cholestatic drug injury was rather benign. Should hepatic panel monitoring in a clinical practice be supported. Most people in the field say no, as we heard very strongly this morning but clearly some sort of monitoring may be valid. If you think you need to monitor the clinical trial, then why don't you need to monitor the patients on the drug post-marketing but what monitoring? Should it be symptoms, like looking in the mirror as John suggested, or something else. We don't know? And should we tailor that to the most likely drugs that cause hepatotoxicity?

And the importance of causality assessment to verify that the drug is the cause of hepatotoxicity is critically important. If you find a case with malignancy in the liver, that's easy but there are other causes we do not know and we may not hear about from the patient as why did they get the toxic reaction, and again one doesn't want to throw the baby drug out with the non-baby bath water. And I think that's all I have to say and I – thank you.

(Appause.)