#357

Experiences as an expert: Educating people while under oath – 25 years since the Sixth International Conference of Racing Analysts and Veterinarians (Hong Kong)

T. Tobin and J. Gutierrez

The Maxwell H. Gluck Equine Research Center, University of Kentucky, Lexington, Kentucky

ABSTRACT

The authors review 25 years of scientific/research expert contributions since the 1985 Hong Kong 6th International Conference of Racing Analysts and Veterinarians presentation. These contributions occurred in the context of greatly increased testing sensitivity, based on ELISA testing in the late 1980s and more recently on LC-MS-MS testing, and principally involve the regulation of therapeutic medications. The regulation of furosemide in the United States is based on some early regulatory threshold work from our group, and is of particular interest in view of the recent South African research establishing the prophylactic efficacy of furosemide. Therefore, the development of furosemide regulation is reviewed, as is the introduction of ELISA testing. In 1994 an international workshop in Lexington validated the concept of "limited sensitivity testing" for a subset of 50 therapeutic medications. Research started on regulatory thresholds for these therapeutic medications, and work on defining regulatory thresholds for these therapeutic agents remains an ongoing challenge. Because of the increased sensitivity of drug testing, regulation of therapeutic medications through use of plasma/serum thresholds has become increasingly accessible. With respect to regulatory thresholds, our research has more recently focused on the need for certified reference standards and stable isotope internal standards for therapeutic medication quantification, a challenge we have been addressing since the mid-1990s.

BACKGROUND

It is 46 years since I graduated in veterinary medicine at University College Dublin and 40 years since I received my doctorate in pharmacology from the University of Toronto. Between 1964 and 1975 I worked on the basic mechanisms of sodium transport and its inhibition by the Somali arrow poison cardiac/glycoside, ouabain. As research training, I can only describe this work as fascinating. I would go into the laboratory in the morning, pull my [Na+K+] ATPase enzyme from the freezer, warm it up, add my ions/ligands/substrates/quasisubstrates/inhibitors/whatevers of choice, in my sequence of choice, and run the experiment.

The experiments were a pleasure to perform. Ouabain binding to [Na+K+] ATPase is highly specific and has a convenient 3-min half-life. Experiments reached equilibrium within 30 mins and only a rare experiment took longer. Experiments would be complete soon and after lunch, data printed out by 4:00 PM, I plotted my data, took them home to Massey College at the University of Toronto and considered the results. Next morning I thawed some more enzyme and asked another experimental question. It was an ongoing conversation with nature, and in a good week you could produce a paper. In fact, in roughly a week I performed the experiments that we published in *Nature*, my first ever senior author publication (Tobin et al. 1970), and a later and even nicer paper, that, in retrospect, I should also have sent to Nature (Tobin et al. 1973).

The *Nature* paper presented evidence that the interaction between the sodium binding sites on the enzyme is "allosteric" that is indirect, and not "steric" or direct. This was a very "cutting-edge" scientific point in 1969, and it was published in as high-profile a journal as one could wish. The report, however, had no immediate real-life consequences; ultimately, it is the practical applications of your research that are of importance; and we would all like to see our research change the way people do things.

Given this circumstance, I was alert for a research area with more real-life impact. In 1974, somewhat unexpectedly, I received an invitation from Dr Jack Bryans to set up an Equine Drug Research Program

in Kentucky. Visiting Kentucky I very much liked what I saw and by January 1975 I was on faculty at the University of Kentucky as an Associate Professor of Veterinary Science and Toxicology.

THE KENTUCKY EQUINE DRUG RESEARCH PROGRAM

As I started in Kentucky, I wondered whether or not my publication rate would decline if I had to catch an actual live horse each morning to start an experiment. Overall, however, we remained active in terms of our output of published papers, although it would be fair to say that the "impact" of the journals in which we published dropped from the *Nature/J. Biol. Chem.* (Sen et al. 1969) caliber of my earlier communications. On the other hand, the real-life impact and technology transfer potential (www.neogen.com; Granstrom and Tobin 1999) of my work was about to increase dramatically. Additionally, from my 10 years of daily conversations with the sodium pump, I had learned three very important things. First, never hesitate to follow your scientific intuitions; second, your experimental results are *always correct* (although your interpretations obviously may not be!); and third, and most importantly, never lack confidence when presenting your interpretations. They were all important lessons.

Another important thing I learned at Michigan State was how to teach. In this area I was assigned Antibiotics, leading directly to the Equine Protozoal Myeloencephalitis patent that David Granstrom and I filed about 20 years later (Granstrom and Tobin 1999). My teaching experience, however, was put to work very much sooner, when I began to make presentations in the matter of the Corrupt Horseracing Practices Act.

THE CORRUPT HORSERACING PRACTICES ACT

The first area in which my experimental work/presentations had significant impact was with reference to the Corrupt Horseracing Practices Act, which I have set forth in some detail in my 1985 paper. The driving force behind this Act was Mr Robert O. Baker, author of a book entitled *The misuse of drugs in horse racing: A survey of authoritative information on medication of race horses* (Baker 1979), whose name will appear again in this narrative. One of his concerns was the use of furosemide in racing horses. When I started in Kentucky, Lasix was widely used in North America, and a pressing question was whether or not it interfered with urinary drug detection. The Lasix dilution effect was relatively easily demonstrated, and it was also established that the effect was over within about four hours (Tobin et al. 1978).

THE US APPROACH TO LASIX

As the pressures generated by the Corrupt Horseracing Practices Act increased, I was invited to make further presentations on this matter. I remember making a number of presentations concerning Lasix and what we knew about it, and stating firmly that the effect on urinary drug testing was over within about four hours. I was vigorously attacked by the anti-Lasix folk for this position, and at one point actually received for review a publication specifically claiming my testimony was in error. To the best of my recollection I returned the paper, indicating my conflict, and the paper was never published in the scientific literature, although my best recollection is that it is published in the Congressional Record. Then, somewhat later, testifying on Lasix before the Illinois Racing Board sometime around 1982 or early 1983 (Tobin testimony 1983), my testimony was interrupted by the Chairman of the Illinois Racing Board, announcing a research "breakthrough". The breakthrough was that research had been performed by Drs Sams and Maylin definitively establishing that the dilution effect of furosemide following the American Association of Equine Practitioners (AAEP) recommended dose of 250 mg IV was over within four hours, confirming in testimony what I had been stating for some time.

In retrospect, it would be fair to say that I was not displeased with this outcome, because nothing in science is ever completely black and white, and while the Lasix effect is for all practical purposes over within four hours, it may not be completely over for all medications.

THE US REGULATORY THRESHOLDS FOR LASIX

The outcome of these events was that in North America Lasix was approved for use in many jurisdictions, subject to Lasix horses being treated and held under supervision in a detention barn for four hours prior to post. This was an expensive and cumbersome solution, and my next expert contribution on Lasix was in response to a request from President Edward Flint of the Kentucky Horsemen's Benevolent and Protective Association to identify a regulatory threshold for Lasix equivalent to the four-hour rule. We performed this research with Dr Sylvia Chay in my group, using, I might add, no fewer than 49 horses, and came up with a mean plasma concentration of furosemide at four hours post administration of about 9.5 ng per mL, and 1/1000 cut-off of about 30 ng per milliliter (Chay et. 1983).

These data were published in 1983 (Chay et al. 1983) and somewhere around four to five years later I gave a presentation on these findings to the Oklahoma Horse Racing Commission. They soon voted to put this plasma threshold into place in Oklahoma but, being good practical administrators, they raised the

regulatory threshold to 60 ng/milliliter. Since then, increased to about 100 ng per milliliter and linked to a less than 1.010 or thereabouts urinary specific gravity, as introduced by Dr Richard Sams in Ohio, this threshold has essentially become the national rule for furosemide regulation in the United States (Harkins et al. 2000).

I might also make the point that my contributions on Lasix were simply descriptive. I just reported the period of time for which Lasix might significantly influence drug detection, and performed some basically descriptive research that yielded a regulatory threshold for furosemide. These were nothing more than simple scientific answers to questions before the racing community, all published in the refereed scientific literature and, if requested, formally presented.

In some quarters, however, my contributions were seen rather differently. In about 2002 Bill Heller went to the trouble of devoting much if not all of his book *Run, Baby, Run* (Heller 2002) to these Lasix matters, in which he presents the entire furosemide matter in the United States in a less than flattering light, and which unflattering light seemed to me to include my research and other contributions (Heller 2002). Now, toward 30 years after my original contributions, I have been very pleased to see an impeccable research contribution from our South African colleagues (Hinchcliff et al. 2009), which clearly establishes the prophylactic efficacy of pre-race administration of furosemide in reducing the incidence of EIPH. This South African work goes a long way towards validating the entire furosemide regulatory process in the United States and, I would also like to think, my now long-ago scientific and expert contributions in this area.

THE ADVENT OF ELISA TESTING

Our next major contribution came in the area of ELISA testing, at its time in the late 80s a dramatically significant advance in the technology of high sensitivity drug screening (McDonald et al. 1988; Tobin et al. 1988). As the ELISA technology came online, and significant numbers of horsemen in certain jurisdictions were being penalized, my wife Vicki was advised, only half in jest, never to start my car in the morning, because to do so could be extremely dangerous. At one point I went out to present on behalf of the Commission at a hearing in New Mexico, where my understanding was that approximately 40 horsemen had been penalized, and I still remember the care with which I maintained an extremely low personal profile during that visit to New Mexico.

In another ELISA-related incident, at about this time we had acquired access to about 50 California post-race samples, ELISA screened them, and identified a small number of cocaine/benzoylecgonine ELISA "positives". Given these ELISA "positives", we volunteered to perform a confirmatory analysis on these samples to support or reject the ELISA data. For reasons that remain unclear, the State of California took a dim view of this request, and I was personally given to understand that we were being accused of trying to extort the State of California, an unexpected research project outcome. In any event, I soon found myself flying out to California to make a presentation before the California Horse Racing Board (CHRB) concerning this matter. My appearance before the CHRB apparently reduced the temperature somewhat, although subsequent mass spectral analysis of the 50 samples established, as close as I can recall, that the ELISA positives were due to traces of parent cocaine, consistent with post-collection contamination.

THE MATTER OF POLICE "WALK-AROUNDS"

At one point during these proceedings I recall finding myself in a one-to-one conversation with the then the Executive Director of the CHRB. The Director, whom I understand had previously been a police officer, took me aside and carefully explained to me some aspects of police power, specifically describing a police "walk-around". A police "walk around" occurs when an officer has stopped a driver for some reason, and it was then explained to me in detail how, as a police officer, he could choose to walk around the car and ticket the driver for every legal infraction known to man under the laws of the State of California. The message received by this 'ivory tower professor' was clear – that the Executive Director of the CHRB was fully familiar with police power and knew precisely when, where and how to use it.

Sometime thereafter I found myself acutely recalling this conversation as I was sitting on a plane in Lexington, waiting to take off for California to assist the defense in a CHRB matter. I decided that my most prudent course of action was to at once get off this particular airplane, which I did, creating what by modern-day standards was only a very minor fuss, and called the lawyer involved. He understood my circumstance and forthwith got a Judicial Order specifically prohibiting, as near as I can recall, anybody from the CHRB, or anybody in the racing business, from interfering with my visit to California in any way, or from taking any post-hearing retaliatory actions against me.

This court order eased my mind considerably, and I went out to California and attended the hearing. More interestingly, these events occurred at about the time that we were bringing the ELISA tests online in Kentucky, one of the more turbulent times in my professional career. Given these circumstances, I occasionally pondered activating the California court order with respect to some events taking place in Kentucky. In the final analysis, however, I chose to just lie low, metaphorically hiding under my office desk and waiting for the turbulence to pass, which it did.

THE ILLINOIS HOOVED ANIMALS HUMANE SOCIETY LETTERS

My next significant episode of expert activity was in my own defense, not a particularly happy position for an expert to be in. In 1994, with broad industry support, we held an international workshop at the Gluck Equine Research Center in Lexington on the matter of "Testing for Therapeutic Medications, Environmental and Dietary Substances in Racing Horses" (Tobin et al. 1995) and we followed this workshop with a 1995 short course setting forth the application of regulatory thresholds in therapeutic medication regulation. Following this short course, I found myself, along with Dr Richard Sams of Ohio and Dr Bob Jack of California and several other colleagues, as the unhappy targets of an anonymous slanderous letter campaign accusing us of foisting a "fraudulent" regulatory concept on the racing industry. These letters had the effect of making the regulatory concept of "thresholds" politically "radioactive", and also made us, personally, equally "radioactive."

Shortly thereafter, these letters, on the letterhead of the Illinois Hooved Animal Humane Society and signed by the Executive Director, Mrs Donna Ewing, arrived in the office of the President of the University of Kentucky, with copies to members of the Board of Trustees. I soon discovered that a signed letter was an entirely different matter from an anonymous letter, and shortly thereafter found myself in the office of the Vice President for Research, along with a University attorney. In the ensuing discussions, it was carefully explained to me that it would be a very good idea for all concerned if I were to sue the Illinois Hooved Animal Humane Society (IHAHS) for slander, and I was reassuringly informed that the University of Kentucky would be "behind me" all the way.

I considered my options and filed suit. It turned out that the letter had actually been written by my old friend, Mr Robert O. Baker, who had 15 years earlier promoted the Corrupt Horseracing Practices Act. To the best of my recollection, Mr Baker had apparently talked Mrs Ewing into signing the letter, and mailing it out on the letterhead of the Illinois Hooved Animal Humane Society. On my side of the fence, a no-cost contribution from the University of Kentucky was a certification by the appropriate University committee that as far as the University of Kentucky was concerned, my activities relevant to the research proposals and publications were entirely correct and appropriate.

Following the Vice President's suggestions, I filed the suit and the legal process started. It turned out that the Illinois Hooved Animal Humane Society was insured to the tune of $1 million, and the insurance also covered my good friend Mr Robert O Baker. We worked away on the matter, and it soon came to the point that I was about to be deposed by the defendants. At this point I again considered my options, we had a major Chicago law firm with $1 million to spend cross-examining me, at which point I forthwith formulated an expert opinion that discretion was clearly the better part of valor, and I quietly dropped the suit. That was the end of this matter with, of course, the minor detail that I had to pick up my attorney's fees. On the other hand, the positive outcome for racing was that my filing suit apparently reduced the appetite of Mr Baker, Mrs Ewing and presumably others for the slanderous letter approach; to my knowledge there have been no similar attacks on the medication policies of US horseracing or any of the individuals involved in these matters since then.

REGULATORY THRESHOLDS FOR THERAPEUTIC MEDICATIONS, ENVIRONMENTAL AND DIETARY SUBSTANCES

While handling this IHAHS suit, my research group was also vigorously pursuing thresholds research, and we published our results in this area in the late 90s, including an invited scientific review of our contributions in 1999 (Tobin et al. 1999). Since then, a considerable portion of my professional activity has been in the area of regulatory thresholds for therapeutic medications, environmental and dietary substances. The ease with high sensitivity ELISA testing allows traces of caffeine, morphine and benzylecgonine to be identified in horse urine immediately gave rise to a need for regulatory thresholds for these substances. In this area, I have also written a series of reviews (Carmago et al. 2005; Buudharaja et al. 2007; Spencer et al. 2008; Carmago et al. 2006), presented at the American Association of Equine Practitioners Meetings and published in the meeting proceedings.

One of the higher-profile events in this area involved the 30 or so morphine identifications reported in English and Irish racing in 2002–03. These identifications were apparently associated with shipment from Amsterdam of groundnut meal containing *Papaver somniferum* fragments to an Irish feed company; an interesting and entirely appropriate outcome of this sequence of events was the introduction of a defined regulatory threshold of 50 ng per milliliter in urine for morphine glucuronides in British racing (Camargo et al. 2005).

UNEXPECTED EQUINE DRUG METABOLITES: THE US AMINOREX IDENTIFICATIONS

Scientifically, a most interesting recent sequence of events with which I have been involved is what I will call the aminorex/pemoline positives in US and English racing. In the United States, starting in Ohio in about July 2005 or thereabouts, a sequence of about 28 aminorex identifications commenced. Soon thereafter similar identifications were reported in Pennsylvania, where the number eventually totaled

about 36, followed by similar sequence of about 18 identifications in Ontario.

At first the source of these aminorex identifications was completely unknown, but in or about March 17, 2007 they were linked to therapeutic administrations of the anthelmintic and immune stimulant levamisole. The critical connection in this area was made by Dr Frank Pellegrini of Freedom Health LLC, Aurora, Ohio, USA, who identified the source of many of these identifications as being innocent administrations of levamisole (Pelllegrini 2010). In his own words, Dr Frank Pellegrini said,

> friends, colleagues and erstwhile clients have sought my help when caught in the aminorex test 'trap.' It has made absolutely no sense that reputable trainers would deliberately use a class 1 substance, where the recommended penalty effectively results in a loss of livelihood; much less continue to do so when a valid test exists for this drug. For two years, I have attempted to identify a common thread. My investigations became more urgent as progressive rumors circulated that Succeed could be the culprit. Finally, in January, I believed I had identified this common factor. It then became imperative that any testing be independent, by the most capable team in the United States. I therefore approached Petra Hartmann with my concerns, and asked that she test Succeed, and my suspect materials. I chose the Industrial Laboratories inc., because Mrs Hartmann is the vice-chair of the Association of Racing Commissioner's International Testing Integrity Program. We received the final report today. I am delighted that we can be a part of resolving this matter for the overall benefit of horseracing in both the US and Canada.

Dr Pelligrini's investigation had revealed that levamisole is metabolized by the horse to aminorex.

Simply put, this was a remarkable piece of scientific/ veterinary detective work, and one that solved a very pressing industry problem. In Kentucky we had been approached on behalf of the Pennsylvania Horsemen, and I brought the matter of the mechanism of the levamisole/ aminorex chemical transformation to my synthetic chemistry colleague Dr Rodney Eisenberg, who rapidly produced the proposed mechanism for the transformation of levamisole to aminorex set forth in Figure 1 below, a sequence of transformations largely confirmed by the recent work of Ho (Ho et al. 2009) in this area.

Identification of the apparent role of levamisole in the aminorex identifications was critical. In Ontario the linkage with levamisole administration resulted in a rapid diminution of the number of positives, although the source of one small subsequent sequence of aminorex identifications in Ontario remains unclear. Research in Pennsylvania performed by my good colleagues Dr Larry Soma and Dr Cornelius Uboh (Ho et al. 2009; Soma et al. 2008) led to re-evaluation of the forensic significance of the aminorex identifications in Pennsylvania. In closing, Dr Pellegrini's very astute clinical identification of the linkage between Levamisole administration and the aminorex positives, and the chemical relationships between these substances represented a major step forward in the determination of the source and therefore the forensic significance of this highly unusual sequence of aminorex identifications.

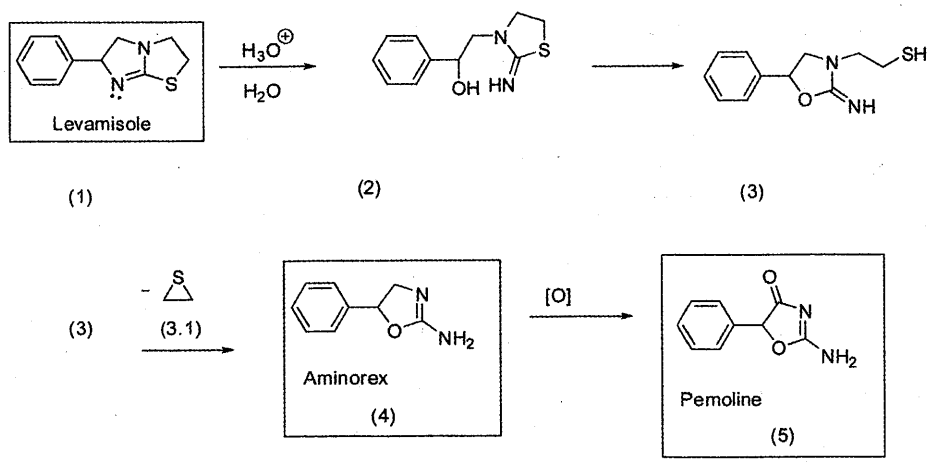

Figure 1: Postulated mechanism for the conversion of Levamisole into Aminorex into Pemoline. Under aqueous conditions Levamisole (1) can be protonated by H3O+ to form intermediate (2). The secondary alcohol in 2 can alkylate the double bond to form the neutral epoxide (3). The sulfur itself can be the possible nucleophile to lose the thiirane (3.1) to form Aminorex (4). It is known that Aminorex can undergo biological oxidation [O] to form Pemoline (5). [Eisenberg 2008, Ho 2009].

A SECOND UNEXPECTED METABOLITE: PEMOLINE IDENTIFICATIONS

The next sequence of levamisole related events took place in England, where in mid-to late 2009 two pemoline identifications and one Tetramisole identification were made on levamisole-treated horses. I was approached by a colleague of the trainer involved, and asked whether or not these pemoline identifications could be associated with levamisole administrations. Review of the chemistry involved by my colleagues Dr Julio Gutierrez and Dr Rodney Eisenberg rapidly extended the previous levamisole-aminorex transformation scheme to include a relatively straightforward oxidation step to pemoline (Spencer et al. 2008). Additionally, since levamisole is the levo isomer of Tetramisole, it is entirely unclear whether or not the Tetramisole "identification" reported was actually a levamisole identification, because special separation techniques are required to distinguish between the very closely related levamisole and Tetramisole (Guterrez et al. 2010).

In closing, it has been quite clear over the years that in an evolving scientific, forensic and regulatory area such as racing chemistry and racing regulation, expert presentations are an integral and important part of the overall process. In the absence of expert presentations, scientifically correct and socially important viewpoints might not be taken into account, as was most dramatically shown in the matter of the "Corrupt Horse Racing Practices Act" and over an entire 30-year period in the matter of the recent scientific validation of the use of furosemide in the prophylaxis of EIPH. In making these presentations I try to keep my material as narrowly focused as possible, since the matters involved are scientific, and I am always aware that the individuals on the other side of the process are, as am I, fulfilling their assigned responsibilities to the very best of their abilities. Along the way, it has been a source of some pride to me that my efforts have helped advance the science of therapeutic medication regulation. Additionally, and equally importantly, our activities have, through a number of intellectual properties developed – the ELISA tests, the EPM patent and some properties hopefully still being developed – contributed to the technology transfer process from the University of Kentucky and, as such, to the economic well-being of my adopted home state, Kentucky.

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WV; Florida; Indiana; Iowa; Kentucky; Louisiana; Michigan; Minnesota, Nebraska, Ohio, Oklahoma, Ontario Canada, Oregon, Pennsylvania, Tampa Bay Downs, Texas; Washington State; and West Virginia Horsemen's Benevolent and Protective Associations, the Kentucky Horse Racing Commission, the Kentucky Equine Drug Research Council, the Neogen Corp. and FrontierBiopharm LLC, and this support is gratefully acknowledged.

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