The History of Automatic Blood Sampling at BAS

Peter T. Kissinger, Ph.D.
Chairman and CEO

Bioanalytical Systems, Inc.
2701 Kent Avenue
West Lafayette, IN
47906-1382

Email:
pete@bioanalytical.com

The development of the first commercial robotic blood sampler is reviewed with respect to why it was needed and the selection of key components which made it possible.

Our principal business strategy at BAS is not a secret. We don't get involved in pure drug discovery research which we see as a very crowded space that has properly attracted enormous investment of late.

The terms of discovery include *genomics, proteomics, high throughput screening, combinatorial chemistry* and *bioinformatics*. Those areas have been extensively covered in many trade publications. From all of these activities comes a tidal wave of new chemical entities (NCEs) to preclinical research. This is where we at BAS begin to focus: combining analytical science and animal science to assess safety and effectiveness before an NCE goes "first time in man."

We are well positioned to add value to the intellectual property portfolios of discovery companies. Those NCEs that pass muster at the preclinical stage (and clearly most will not) still perhaps have only a 1 in 10 chance of becoming a useful drug, but that's a lot better than the 1 in 1,000 chance that is the case entering the preclinical chute. This is clearly a process by which "interesting technology" becomes "investment grade technology," deserving a more serious look. The primary focus of preclinical *research* is to evaluate candidates in laboratory animals. Preclinical *development* then adds other components such as scale-up for synthesis and work toward a viable formulation.

In Vivo *Electrochemistry*

Our involvement in this topic goes all the way back to 1972 when I first began discussing the idea of implanting electrodes into animals for real-time monitoring of neurotransmitter release in brain tissue. Prof. Ralph Adams of the University of Kansas got me engaged in this thought. I was very skeptical. Most scientists are that way. We prefer to have some facts, some data. On the other hand, the very notion of real-time monitoring of chemical events in the tissue of a conscious, freely moving mammal was, for me, awesome. It remains awesome! In 1972, this was viewed as impossible. Science fiction. Pigs will fly first! Over the intervening 30 years, a great deal has been accomplished with *in vivo* electrochemistry; however, selectivity and concentration detection limits inhibit its use for all but a few analytes.

Microdialysis

The possibility of *in vivo* monitoring evolved further in 1984 when I first began conversations with Prof. Urban Ungerstedt of the Karolinska Institute about commercializing *in vivo* microdialysis systems for neuroscience and other applications. It was an early point of interest to note the relative advantages and disadvantages of the dialysis approach for quantitation of endogenous metabolites and drug substances in a variety of biological fluids. To be brief: microdialysis provided a protein free stable sample (no enzymes) that was immediately compatible with existing BAS bioanalytical approaches. The technique also provided no fluid loss from any tissue or compartment. On the other hand, the dialysis process was dependent on physical chemical properties (size, charge, hydrophobicity, diffusion coefficient) and presented certain ambiguities with regard to quantitation. Dialysis is also a pain-free means of sampling and provides for excellent time resolution vs. traditional methods. It is also particularly easy to automate. My enthusiasm for microdialysis remains high.

Since microdialysis was first commercialized in 1985, it has become increasingly evident to our scientists that the commercially attractive applications would focus on small hydrophilic molecules such as glucose, neurotransmitters, amino

acids, small peptides and hydrophilic drugs. At BAS, our primary focus is drug development. We could clearly see microdialysis as a pharmacodynamic tool, but its use for pharmacokinetics was (in a commercial sense) limited by quantitation issues, by the fact that it is experimentally demanding and by the fact that more and more new drug substances were increasing in molecular weight and hydrophobicity, both of which present technical challenges to microdialysis. While a gallant effort was made to explore microdialysis for pharmacokinetics (which we saw as a potentially larger market than neuroscience) and for diabetes (glucose), our customers resisted these attempts.

Blood Sampling

As the years went by, it became increasingly clear to me that we needed to fit with pharmaceutical research tradition and be able to sample blood using the same automation philosophy we earlier applied to microdialysis.

Why blood? A few reasons: tradition; total drug concentration (protein-bound as well as free); the importance of hematology in drug development; protein-based drugs are increasingly important and need to be sampled; endogenous marker substances are important; clinical chemistry is important; lipids are interesting, too, and so are viruses.

We wanted to look not only at blood, but also at bile, spinal fluid and other more exotic possibilities in both awake and anesthetized rodents and restrained or suspended animals, including dogs and primates. Our feeling was that sampling blood in parallel with microdialysis would be a marvelous advance in monitoring animals pain free and under low physiological and psychological stress.

As noted earlier, BAS focuses on preclinical development and not discovery. We could see an enormous increase in new chemical entities coming from discovery that would hit a bottleneck at the laboratory animal level. We also envisioned a need to reduce the number of animals used to get data and to refine experiments to improve data quality. BAS subscribes to the three R's of research animal models: refine, reduce, replace.

Often we would see different departments in a large pharma doing different experiments with the same candidates for purposes of observing behavior, physiology, pharmacokinetics, blood/brain barrier transport and acute toxicology. We envisioned bringing some of these tasks together to improve throughput, at least at the screening level, using common apparatus and software. We recognized that this was hard for big pharma to digest because of the way they are organized, but small companies with more focused objectives (fewer therapeutic goals, fewer NCEs, fewer boundaries between disciplines) could implement these ideas with some efficiency, as could we as a preclinical contract research organization (CRO) having tremendous assets in both animal science and analytical science.

The above thoughts defined the Culex® project. Now it was a matter of how to go about it! Various academics and a few companies had tried automatic blood sampling over the years. We were especially intrigued by the work of Professor I.C.A.F. Robinson of the MRC in the UK, who was willing to communicate with us (1), as were six major pharmas who contributed ideas to the project. Prof. Robinson demonstrated feasibility in an academic sense, but he did not have the resources needed for high precision, very low dead volume and flexible protocols.

The early published and unpublished attempts at automatic blood sampling basically employed the following components:

1. A peristaltic (or "roller") pump
2. A liquid swivel above the cage to permit animal motion
3. A rotated valve with electrical actuator
4. A digital timing device to start/stop the pump and actuate the valve
5. A fraction collector

It is not hard to cobble together a working system from these five components. This is especially so after seeing what the pioneers had prototyped. This general approach will work to some degree, but has serious weaknesses.

In our studies of the technology, including prototyping several versions in the laboratory, we came to the following conclusions:

1. A peristaltic pump is a very bad choice for accurate delivery of small volumes. It is likewise not ideal for small diameter tubing that minimizes system dead volume. We opted for a syringe pump where every motion of the pump is precisely controlled by software and where there is little "softness" in the system due to tubing compression and expansion or stretching of tubing over time. Peristaltic pump performance drifts over time. We wanted a system that would hold calibration for many years.

2. A liquid swivel is a very bad choice because of clotting potential, leaking potential, high maintenance, and difficulty with sterilization resulting in likely bacterial contamination on internal parts. Several of the "home made" swivels we used had substantial dead volumes (as much as 100 µL or more).

We wanted to be able to carry out simultaneous microdialysis experiments and/or physiological measurements and/or drug infusions and/or biosensor experiments (*a la* glucose) with lines running to the same freely moving animal. This is absolutely impossible with a mechanical swivel or commutator.

We also were required to provide for drug infusion simultaneously with blood removal. Either a bolus injection; repeated, timed injections; or continuous infusion needed to be accommodated. A swivel presents the very real risk of cross contamination between drug infusion and

blood collection. Such contamination is a bad laboratory practice (BLP).

We are very familiar with swivel problems. We have sold swivels for sixteen years. In fact, we still sell them. They have some practical uses, but the Raturn® technology supersedes their use for most purposes, even for microdialysis. This patented technology is in very widespread use, but those not yet familiar with it can refer to the references (2, 3).

3. A rotating valve is a very bad idea. Dead volume and sterilization are both issues. It quickly became apparent to us that a single uninterrupted tubing set that could be fully sterilized with only a single connection at the animal catheter was very desirable.

4. A computer provides far more flexibility than a hardwired timer, and allows for set protocols, printed reports and integration with behavioral and ultimately physiological information from the same animal on the same time clock. We anticipated the possibility, for example, of video monitoring of animal behavior, remote monitoring of the system via internal networks and even the Internet. We anticipate the role of broadband Internet access, including wireless, over the next few years. As that evolves, Culex will evolve as well.

5. Our 16 years of microdialysis work gave us plenty of experience with accurate collections of even sub- 5 µL samples with multichannel micro fraction collectors of various designs. This experience made it straightforward for us to utilize a dual channel collector with rigorous maintenance of vial temperatures below four degrees. Other fraction collectors have not used capped vials which are essential to prevent evaporation. We also use the vial septum to wipe the needle to prevent cross contamination between vials.

6. We chose to use a 4-animal cart with a symmetrical square configuration rather than a linear or bench top configuration. Why? Most animal facilities are already designed for carts and can be hose washed. Carts are easy to move and can be arranged with high density for longer-term experiments. Our vision was that two carts could feed one 96-well plate with samples from 8 animals over a 24-hour PK screen.

7. We realized that one of the weaknesses of existing blood sampling systems was the extensive time and effort required to physically set up and prepare these systems each time a new study was begun. This procedure includes connecting and purging fluid lines, removing unwanted air bubbles, checking for catheter patency, etc. We worked to minimize the setup requirements and make them as easy as possible.

We realized these preparation steps require flexibility and are best handled by the operator while at the individual blood sampling station. For this reason, we provide a number of individual function keys right at the blood collection station, as well as feedback to allow the operator to prepare each station and see immediate results without a need to access the computer. This is one of those features that people don't appreciate until they actually use the equipment.

8. It was likewise deemed absolutely essential that the sealed fraction collector vials be compatible with 96-well plates so we could take advantage of the considerable investment we had made in robotics for processing samples in plates, including centrifugation, 96-well solid phase extraction, and 96-well autosampling for LC/MSMS.

The purpose of the above is simply to describe how we arrived at our Culex technology (3-6) and where we can go next. Like Bill Gates, I ask our clients, "Where do you want to go today?" and also, "Where do you want to go tomorrow?" (Sampling from a transgenic mouse perhaps? Or sampling spinal fluid? Or bile?)

When great minds are collectively put on a subject, fantastic things can happen. This project has now involved over 100 scientists, engineers, ADME-PK scientists, DVMs and veterinary technicians. We continue to collect ideas and advance the possibilities to improve throughput in preclinical drug development.

With every advance in technology, there are unexpected consequences. When Culex was originally developed, we focused on labor savings and accomplishing several objectives such as PK and PD simultaneously in the awake rat. Those objectives remain valid. Far more important, however, is the recognition that stress of conventional sampling from awake rats dramatically influences the data for many drugs. The physiology (blood pressure, blood flow) and biochemistry (stress hormone release, metabolism, even protein expression) vary significantly among anesthetized, awake but stressed (cannulated or not), and awake cannulated animals sampled stress free (without restraint and human handling). The latter situation provides the most consistent and meaningful data for scaling to larger animals. This has brought our clients from an original perspective that "automated blood sampling is nice to have" to a new perspective that "automated blood sampling is something we must have to get the best data."

References

1. *R.G. Clark, G. Chambers, J. Lewin and I.C.A.F. Robinson, J. Endocr. 111 (1986) 27-35.*
2. *Handbook of Microdialysis, Bioanalytical Systems, West Lafayette (1997) 45-47.*
3. *www.culex.net*
4. *S. Peters, J. Hampsch, M. Cregor, C. Starrett, G. Gunaratna and C.B. Kissinger, Current Separations 18:4 (2000) 139-145.*
5. *C. Bohs, M. Cregor, G. Gunaratna, W. Schmidt and C. Kissinger, Current Separations 18:4 (2000) 147-151.*
6. *P.T. Kissinger, Genetic Eng. News, 21:18 (2001) 34.*