Biotech Start Up Companies

In this issue we have included a review paper from one of our sister journals, *Technovation*: "Technological Infrastructure and Regional Economic Development of Biotechnology Firms" by Peter Haug and Philip Ness. The paper describes empirical evidence from 37 commercial biotechnology firms on background attributes, company origins, financial capital resources, market distributions and strategic alliance. Data were collected through extensive personal interviews with senior executives and scientists. This paper contributes evidence on the origins and operations of commercial biotechnology firms in the state of Washington.

Whilst at first glance it may seem a little odd to have included a review of US biotech start-ups in a journal dedicated to biosensors, I have my reasons. I am keen to feature new start-up companies in the biosensor field, knowing that it is likely that many of you would like to have a "start-up" company of your own. This is, of course, one of the most frequent types of "little acorn" from which the "mighty oaks" of commerce grow.

There have not, I believe, been any such studies solely concerned with biosensor start-ups - we hope someone will do one so we could publish it! Until there is, I thought it would be quite instructive for readers to find out how their erstwhile brethren in biotech have been faring in the Washington area of the USA. Whether this mode of business start-up and its success (or failures) is in any way indicative of moves in other related technologies or of other geographic areas it is difficult to tell. The review should give some indication of the prospects ahead for any would-be entrepreneur in biosensors. It was not my intention to discourage, but maybe the pass rate shown here could provide salutary lessons and help avoid costly mistakes.

Biosensors & Bioelectronics Update

Roy Szweda

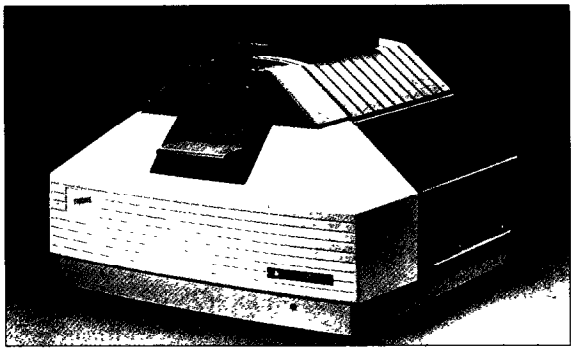
In this international update on new developments in biosensors and related activities, we report on applications of biosensing technology to food processing and environmental monitoring. Plus a look at a new impedance "biosensor" for the study the shape and movement of mammalian cells. We begin, however, with an update on the FAST IASys.

UK - FAST IASys makes FT

It was with some satisfaction that we read a short article on the IASys instrument (see photo) in a recent issue of the *Financial Times*. As readers may recall, we published a comprehensive look at the instrument in issue 7/8, of *Biosensors and Bioelectronics*, last October.

The article, written by Clive Cookson, was entitled "Catching up with bio reactions". It reports on the use of an IASys system by Arnold Coffer who runs the Imperial Cancer Research Fund's protein isolation and cloning lab in London. He says that it allows him to "obtain within minutes information that would previously have required days of experiments with test tubes, using radiotracer reagents".

0956-5663/94/$7.00



Dr Coffer's IASys is utilised in a number of research projects. The article mentions one example: the evaluation of new compounds which block the action of oestrogen hormones as treatments for breast cancer. "The copies of protein which serve as the oestrogen receptor in human cells are made and then we see how strongly different drugs bind to it". Dr Coffer said, "I don't think one machine will be sufficient here, now that word is getting around about what it can do. Maybe two or three would satisfy the requirements of the ICRF."

Meanwhile, FAST has reaffirmed its commitment to biosensor interaction analysis with a new worldwide sales and service network for the IASys microcuvette system.

The IASys is now supported in N. America by a FAST service, sales and marketing operation in Paramus, New Jersey, USA. This new operation includes a well-equipped lab and demo facilities. Europe, Australia and the Far East will be supported by Fisons Instruments.

Contact: David Fortune, Fisons Applied Sensor Technology Ltd., Saxon Way, Bar Hill, Cambridge CB3 8SL UK. Tel/fax: [44] (0)954 789976 / 789417.

Ireland - FLAIR biosensors for detecting bacteria

In the European Community's FLAIR (Food Linked Agro-Industrial Research) programme, a shared-cost project with four partners is ongoing on the development of an acoustic biosensor to detect food spoilage and/or pathogenic bacteria.

It is intended that the biosensor reduce the time of microbial detection and increase the sensitivity and specificity of detection thereby enhancing food safety. The project has three components: biorecognition component development, the immobilization of biorecognition components on sensors surfaces; the development of an acoustic wave transducer for biosensor applications.

The biorecognition component (antibodies) development involves raising antibodies against bacteria in a strain or species specific manner and to date antibodies have been isolated and raised against a receptor protein for *Pseudomonas* species strain M114. Such antibodies were shown to react with whole cell preparations of *Pseudomonas* species strain M114.

Having selected antibodies for use in a biosensor, the question needs to be addressed as to how to attach the antibodies to the sensor surface. This has involved studies of antibody immobilization on sensor surface materials by physical absorption and attachment through chemical modification of the antibodies. Surface materials have been chosen which are deemed to be the most appropriate for the intended use of the biosensor. Radioactive labelling is being used to monitor antibody absorption to these surfaces. Occupation of the entire sensor surface with an even layer is of utmost importance to the operation of the sensor, as non-specific binding would alter the signals from the sensor and give inaccurate results.

Sensors based on acoustic wave transduction operate by sensing the mass of the analyte bound to the sensor surface through an interaction with an elastic wave probe. Acoustic transducers do not require that the analyte possess any special properties such as optical, electrochemical or enzymatic activity. Acoustic wave devices are therefore a class of transducer which shows considerable potential for use in low cost sensors applicable to many types of assay. Two prototype acoustic transducers are currently being utilised to optimise a complete biosensor design. In particular, work is now focused on the development of suitable packaging technologies and optimised multi-channel transducer

configurations to improve the stability of the final biosensor.

Contacts: Prof. F O'Gara, Dept Food Microbiology, University College, Cork, Ireland. Tel/fax: [358] (0)21 276871 / 275934.

National Network Leader is Dr Suzanne E Emmett, Leatherhead Food Research Association, Randalls Road, Leatherhead, Surrey KT22 7RY UK. Tel/fax: [44] (0)372 376761 / 386228.

F-FE Project Leader is Dr TR Gormley, The National Food Centre, Dunsinea, Dublin 15, Ireland. Tel/fax: [358] 383222 / 383684.

UK - Biotrace joins Campden project

Biotrace Ltd. has been appointed the exclusive supplier of ATP-based hygiene monitoring equipment in a European research project. The project began in January 1993 and will run for three years. It will develop new test methods to evaluate the cleanability of food processing equipment. With ten partners from seven countries, overall coordination is being handled by the Food Hygiene Dept of the Campden Food and Drink Research Association (CFDRA).

The project includes research labs, equipment manufacturers and two food companies - HJ Heinz & Co. Ltd. and Unilever. Initially, microbial films - biofilms - mixed microbial/food soil films and artificial food soils will be developed and standardised so that a rang of testing procedures can then be evaluated and compared.

The procedures will include the application of ultrasound, image analysis, UV light, mobile sensors for closed process equipment and ATP bioluminescence. ATP can be detected in quantities smaller than 10^{-14} g using bioluminescence techniques, and the project will apply the new ultra-sensitive Multi-Lite system from Biotrace to assess the effectiveness of the cleaning process.

Contact: Lionel Brooks, JDK Public Relations, The Old Granary, Squerryes, Goodley Stock Rd., Westerham, Kent TN16 1SL UK. Tel: [44] (0)959 562772.

CFDRA Contact: Victoria Johns, Campden Food & Drink Research Association, Chipping Campden, Gloucs., GL55 GL0 UK. Tel/fax: [44] (0)386 840319 / 841306.

Sweden - Lactic acid in pork as a freshness indicator

In *Analytica Chimica Acta*, (283/2 (727-737), 1993), Shu H.-C. Hakanson H. and Mattiasson B. of Lund University, Sweden, report on "D-Lactic acid in pork as a freshness indicator monitored by immobilized D-lactate dehydrogenase using sequential injection analysis".

The authors describe how a sequential injection analysis system was set up to quantify D-lactic acid in pork. D-Lactic acid being a useful indicator of metabolism of spoilage organisms, they say. The analytical system is fully computerized and is able to monitor the concentration of the acid in an assay cycle of 160s including 90 s incubation time when the flow is stopped. The enzyme used in the analysis is D-lactate dehydrogenase co-immobilized with L-alanine aminotransferase on porous glass.

The experimental set-up gave good reproducibility down to 0.1 mM D-lactate and the assay could be repeated at least 500 times with the response maintained.

Contact: Department of Biotechnology, Chemical Center, Lund University, P.O. Box 124, S-221 00 Lund, Sweden.

UK - PL-LALS from Polymer Labs

The PL-LALS - dual detector for low angle light scattering systems has many uses in biochemistry and is ideal for the characterization of a wide range of proteins, polysaccharides and nucleic acids.

The PL-LALS is now used frequently as an on-line gel filtration detector to provide molecular weights (MW), which can be obtained quickly without the need for column calibration. It can detect very small quantities of high MW material. Since the scattering light intensity is proportional to the square of the MW, the system is sensitive to

trace amounts of high MW species and therefor provides information on protein aggregation. It has been used, the supplier says, to yield rapid and reliable MW and concentrations of both individual protein units and aggregations.

Contact: Polymer Labs Ltd., Separation Science Div., Essex Rd., Church Stretton, Shropshire SY6 6AX UK. Tel/fax: [44] (0)694 723581 / 722171.

Denmark - Sensors developed for sanitation

A related project under the EC FLAIR programme (see above) is developing a range of sensors (not biosensors in this case) to detect fouling in the inaccessible parts of food processing equipment.

An ultrasonic sensor under test was found to be influenced by product viscosity and probe alignment to such an extent that measurement of fouling films was not possible. As a result, a new transducer type has been developed which should be able to measure fouling film from 0.5-6.0mm thickness.

Studies measuring fouling on a plate heat exchanger are also be conducted using an optical sensor, together with a vibrational sensor which is an integral part of the neat exchanger and has been shown to be capable of detecting fouling layers of milk down to 0.1 mm thickness.

Contact: Dr HO Mikkelsen, Biotech Institute, Holbergsvej 10, PO Box 818, DK-6000 Kolding, Denmark. Tel/fax: [45] 7552 0433 / 9989.

USA - Food Biosensor Book

Food Biosensor Analysis, Edited by Gabrielle Wagner and George G. Guilbault, published in October 1993, ISBN 0-8247-9150-9. Price: $125.00. Published by Marcel Dekker Inc., 270 Madison Ave., New York, NY 10016. Tel: [1] (212) 696 9000.

This book is number 60 in the Food Science and Technology Series published by Marcel Dekker, NY, USA. It is "intended to serve as a practical guide for all people involved in the processing or production of food or in controlling the composition and quality of raw food materials and products", or so say the editors in their preface.

Reviewing technical books is often a tedious task because most would only be consulted when required rather than fully read. This book, however, could be read from cover to cover and the reader would then well feel apprised of the state of the art of this fascinating area of application of biosensor technology. Moreover, the practioner of food technology should find much here to help apply the technology to his/her particular process requirement.

This book may, I suspect, help overcome some of the caution which sometimes greets the suggestion of applying biosensors to the food environment. As the editors point out, there are many cases of "sophisticated biosensors fail[ing] miserably during their first contact with real food". To help overcome this aversion to biosensors, the most successful types are explained by authors who have "hands on" experience. This book will help newcomers to make the best decision as to which biosensor type best suits their special application. Conversely, it should help the biosensor technician ensure that his or her new device is indeed well-suited to the chosen context.

Over ten chapters the editors and other leading names in the field introduce, define, and demonstrate the application of biosensors to food technology. This well-presented and very readable book is fully illustrated and indexed with over 770 literature citations. It would be of great benefit to people coming from either a food technology or biosensors background. There aren't too many really usefully practical books on the application of biosensors around so this one is especially welcome. The editors are to be congratulated in bringing together such a useful and informative collection of chapters. One can but hope that there is someone out there who is planning a similar treatment of other biosensor applications, such as environmental monitoring and patient diagnostics.

USA - FDA proposes controls on medical devices

The US Food and Drug Administration (FDA) is proposing design controls to make current manufacturing standards for medical devices much tougher.

According to *The Wall Street Journal*, the proposed regulation would apply to various medical devices, including heart valves, catheters, pacemakers and ventilators, and medical device software.

Having recalled and reviewed 2 000 different medical devices during the period 1983–1989, the FDA says that about 44% of the problems found were caused by design flaws. The proposal would require an analysis of the potential hazards associated with the use of the medical devices.

Australia - New retinal chip developed

A research team led by engineer Derek Abbott, in Adelaide, Australia has developed a new microchip device which emulates many of the functions of the vision of an insect. Moreover, the new device is claimed to be installed for 10% of the cost of existing vision systems for robots etc.

The device has been successfully tested on an electronic arm which had been programmed to veer away from any approaching object detected by the retina chip. It could see objects and react to them within 10 ms - faster than current vision systems using lens systems like the human eye and which require complex computer processing to interpret every image before the robot can initiate evasive action.

The retina chip comprises a row of 60 light detectors wired to a bank of parallel processing elements and more than 22k transistors all in an area of about 1 cm square.

USA - Biocompatibility of implanted electrode arrays

"The implantation procedure studied produces limited amounts of tissue damage, and the arrays used are biocompatible. Arrays insulated with polyimide over a primer had significantly greater involvement of macrophages, gliosis, and capsule formation than uncoated arrays and arrays insulated with polyimide without primer, indicating a possible reaction to aluminium oxide in the primer."

This report comes from K. Horch et al. in the *Journal of Biomedical Material Research* (27, 11, 1993, p.1393–1399). The passive biocompatibility of silicon-based electrode arrays was studied in the cortical tissue of cats. Three types of arrays were used: uncoated; coated with polyimide; and coated with polyimide over an adhesion promoter. Fifteen arrays were implanted for 24 h to determine early tissue reaction to the implantation procedure, and twelve arrays were implanted for 6 months to determine structural and material biocompatibility.

Oedema and haemorrhage were present around the short-term implants, but involved less than 6% of the total area of the tissue covered by the array. With chronic implants, leukocytes were rarely present and macrophages were found around one-third of the tracks.

Contact: K. Horch, Department of Bioengineering, 2480 MEB University of Utah, Salt Lake City, UT 84112, USA.

Germany - Miniature sensors for water quality

Elbau Elektronik Bauelemente GmbH, Berlin, is developing a series of miniaturized oxygen electrodes for use with water quality sensors. Enzymatic or microbiological reactions within the biosensors consume or produce oxygen or hydrogen peroxide which is then detected by a cell.

Elbau's electrodes have an active surface area of 0.2 mm^2 and a response time of below 6 s. Lifetime is said to be over a year with a signal stability of +/- 1%.

The electrodes have a very regular convex surface - essential for membrane tension and constant electrolyte film. The company says that it is open to custom product design and the first of the range are already in use in industrial systems.

Contact: Silvio Fischer, ELBAU Elektronik Bauelemente GmbH, Strokower Str. 115A, 10407 Berlin, germany. Tel/fax: [49] 30 421 1800 / 423 2711.

USA - Impedance "biosensor" studies shape and movement of cells

A US start-up company has recently reported a new biosensor which can continuously track morphological changes of cells. It is said to be capable of detecting vertical motion of cells in the region of 1 nm by ECIS - electrical cell-substrate impedance sensing. The authors say that this should greatly aid the measurement of the shape and movement of cells which up to now has relied on qualitative techniques such as optical microscopy.

The "morphological biosensor for mammalian cells based on the ECIS method" is described in a recent issue of *Nature*, (Vol. 366, 9 December, 1993, pp 591-592) by two authors, Ivar Glaever and Charles R. Keese (Rensselaer Polytechnic Institute, Troy, New York 12180-3590 USA) who three years ago founded the company Applied BioPhysics Inc. to commercialize applications of the ECIS method. They have been helped in this endeavour by a number of SBIR grants from the NIH.

The apparatus is described as comprising two gold electrodes emplaced on the bottom of a culture vessel: a tiny one and a large gold electrode. The mammalian cells from the culture attach to the small electrode and cause impedance changes. These changes correlate to cell shape and mobility. The impedance measurement is made with a 4 kHz, 1 V amplitude signal applied to the electrodes through a 1 Mohm resistor. A lock-in amplifier and PC data acquisition system enable detection and presentation of magnitude and phase of the voltage across the sample. The system is configured so as to enable continuous measurement for automated analyses.

The work has also examined the effect of adding chemicals to culture media. The authors say that the method could provide an alternative to animal testing of toxic materials. The article illustrates this claim with the effect of thrombin on endithelial cells, murine macrophages to heat-killed *Listeria monocytogenes* and MDCK endithelial cells respond to Triton X. In each case there is a clear response to the polluted media via the impedance sensing equipment.

The authors conclude that this method offers many opportunities for the study of tissue cultures. The quantitative results taken in real-time and continuously, offer distinct advantages over conventional approaches. The authors hope that the work presently undertaken by their own company labs will be taken up by others and the technique be more fully explored and applied.

We hope to return to Applied BioPhysics Inc., in one of Jo Ann McDonald's USA Reports.

Contact: Applied BioPhysics Inc., 1223 Peoples Ave., Troy, NY 12180 USA.

Israel - Update on immobilized enzymes

In one of our sister publications, *Trends in Biotechnology*, (11/11, 1993, pp 471-478), E. Katchalski-Katzir, discusses "Immobilized enzymes - learning from past successes and failures".

The use of immobilized enzymes in the food, pharmaceutical and chemical industries has increased steadily during the past decade. Further research and development in enzyme technology is expected to expand their use in the synthesis of chiral drugs, complex organic compounds and fine chemicals, and lead to the construction of novel, specific biosensors.

Contact: Department of Membrane Research, Weizmann Institute of Science, Rehovot, Israel.

USA - Ambulatory health care markets to reach $7bn by 1998

According to a new report from Frost & Sullivan, Mt View, CA, USA, "as US healthcare moves increasingly out of the hospitals in search of lower-cost, more patient-friendly environments, the market for ambulatory healthcare centre products will mushroom".

The market will grow by nearly seven times from $1bn in 1992 to $6.9bn in 1998, at a 37% CAGR states the report *Alternate Site Acute Care Equipment & Supply Markets: Quest for Cost Reductions Fosters Ambulatory Center Growth.* Code 42054, price: $1695.

Diagnostic instrumentation accounted for 57% of total market revenues in 1992, primary manual medical instruments 23%, medical supplies 17% and diagnostic products and biological substances 3%, says the report. The movement away from hospital in-patient care towards out-patient "alternate site" healthcare will continue strongly through the 1990s and beyond. The move to minimally invasive surgery will continue to facilitate ambulatory centre growth.

As diagnostic products improve, growth factors and genetic engineering will see increased usage. Disposables and one-time instrument packs will continue to gain popularity. Specialised surgicentres as well as other types of ambulatory facilities, are seeing rapid growth and the continuing expansion of these new facilities will drive growing product group revenues. While significant growth is expected in all centre types, the strongest will be those specializing in haemodialysis / haematology, and obstetrics and gynaecology. There are more than 1500 of these ambulatory centres in the USA - little expansion is expected in this number. Competitive factors in this area will include quality and efficiency and provision of ancillary customer services.

UK Contact: Kristina Menzefricke, tel/fax: [44] (0)71 730 3438 / 3343. US Contact: Amy Arnell, tel/fax: (415) 961 9000 / 5042.

Germany - Pesticide immunological FO biosensor

As part of a long-term environmental monitoring programme, the Gesellschaft fur Biotechnologische Forschung mbH (GBF) in Braunschweig, an immunological fibre optic biosensor to detect low levels of pesticides in water has been developed.

The biosensor utilises fluorescent-labelled antibody binding. Triazine antibodies, conjugated with fluorescein isothiocyanate, were detected after binding with the fibre's surface by a reduction in fluorescence. A very high sensitivity of 0.1 ng/ml is said to have been achieved for the pesticide terbutryn.

Contact: Prof. R. Schmid, GBF mbH, tel/fax: [49] 5316 1810 / 1813.

Ukraine - FETs for formaldehyde & glucose

We report on two new publications of work in progress at the Institute of Molecular Biology/Genetics, Academy of Sciences of Ukraine, Kiev, Ukraine.

Firstly from the journal *Analytical Biochemistry* (215/2 (216-222), 1993), Korpan Y.I. *et al.*, have a paper on a cell biosensor for formaldehyde "based on pH-sensitive transistors coupled to methylotrophic yeast cells with genetically adjusted metabolism".

A cell biosensor specific for formaldehyde was developed using double-mutant cells of the methylotrophic yeast *Hansenula Polymorpha A3-11*. The authors say that activities of some of the enzymes in the metabolic pathway of the wild-strain cells were deliberately suppressed by introducing respective genetic blocks to optimize the selectivity and acidification rate. The mutant yeast cells produced in this way were then immobilized in Ca-alginate gel on the gate of a pH-sensitive field effect transistor (FET).

Local acidification of the extracellular medium due to specific conversion of formaldehyde was recorded. The steady-state response time of the biosensor was 2-3 min, i.e., about 10 times shorter than the response time for the alcohol-specific cell biosensors the authors say were described earlier.

The linear dynamic range of the sensor's response corresponds to formaldehyde concentrations of 2 to 200 mM. The operational stability of the sensor was "not less than 4 h" and the biosensor demonstrated high specificity to formaldehyde with no response to several organic acids, methanol, and other alcohols, except for low sensitivity to ethanol.

The influence of sample buffer capacity and pH on the sensor response, as well as thermostability, is also reported in the paper.

Readers are invited to peruse the article on the Drager BioCheck-F commercial formaldehyde monitor in this issue.

In our second report we note another paper from the Institute of Molecular Biology/Genetics, the Academy of Sciences of Ukraine, Soldatkin A.P. *et al.* It is entitled "Glucose-sensitive field-effect transistor with additional Nafion membrane. Reduction of influence of buffer capacity on the sensor response and extension of its dynamic range. This was published in *Analytica Chimica Acta* - (283/2 (695-701) 1993) - and reports how a glucose-sensitive enzyme FET (ENFET) was prepared by cross-linking glucose oxidase with bovine serum albumin in saturated glutaraldehyde vapour on the gate area of the FET.

Nafion membranes were deposited on the top of the glucose ENFET by a spin-coating procedure. Additional Nafion membranes resulted in a substantial reduction of the effect of the buffer concentration on the ENFET's response and in an extension of the dynamic range of the sensor up to concentrations of more than 10 mM glucose. The effects of pH, buffer concentration and ionic strength were examined for the glucose ENFET with and without NAFION membranes.

Contact: A.A. Shul'ga, Inst. of Molecular Biology/Genetics, Academy of Sciences of Ukraine, Zabolotnogo Strasse 150, Kiev 252143, Ukraine.

Australia — FRL & CSIRO biosensors

Improvements to the safety and quality of both food and water will be target areas in the development of prototype biosensors.

The Food Research Laboratory is fortunate to be a participant in the successful submission to the Board of CSIRO for priority funding of a Multi-Divisional Program on Biosensors. The three year collaborative effort will commence officially in July 1994 and is be spread across six divisions: Animal Health, Applied Physics, Biomolecular Engineering, Chemicals and Polymers, Food Science and Technology and Plant Industry.

The final decision on the most appropriate prototypes to use will be made following the completion of an exhaustive market assessment presently underway.

Switzerland - calorimetric sensing in bioanalytical chemistry

In *Trends in Analytical Chemistry*, P. Bataillard describes principles, applications and trends in calorimetric sensing in bioanalytical chemistry.

In the article (pp 387-394, 12/10, 1993) the author describes the great potential calorimetry has shown in bioanalytical chemistry. This is because most biochemical processes involve a change in enthalpy. Two types of approach have been developed:

i. adiabatic calorimetry - this relies on the absence of heat exchange between the reaction vessel and the external environment, and

ii. heat conduction calorimetry - this involves measurement of the heat transferred from the vessel to a surrounding heat sink.

Both principles, with their respective advantages and drawbacks, have been applied to microcalorimetry for the analysis of (bio)chemical compounds.

Immobilization of the biomaterial in the vicinity of, or directly onto a small temperature or heat sensitive transducer has led to the concept of a calorimetric biosensor. In comparison to the traditional calorimeter, the calorimetric biosensor is better suited to continuous monitoring and size reduction. This simplified but sensitive device, the author says, is expected to solve numerous problems in various fields of analytical chemistry.

Contact: Corporate Analytical Research Dept., Ciba-Geigy Ltd., Basel, Switzerland.

B&B is always pleased to receive news items and press releases pertaining to the field of biosensors and bioelectronics. Please mail or fax your news to Roy Szweda, Managing Editor, [44] (0)865 843971.