IN VITRO MEASUREMENT OF GASTRIC MUCUS SYNTHESIS BY A DYE BINDING TECHNIQUE

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Much interest has been shown in recent years on the role of gastric mucus as a cytoprotective agent. The need for a rapid, simple experimental technique to measure the synthesis of mucus is required. The procedure to be demonstrated meets these criteria, being an extension of the method of Corne, Morrissey and Woods (1974) using the dye alcian blue 8 GX.

Male Sprague-Dawley rats (150-200g) are killed by cervical dislocation and the stomachs rapidly removed. The fore-stomach is cut away and the secretory portion opened along the line of greatest curvature and rinsed with saline. The stomachs are incubated, at 37°C, in oxygenated Krebs solution containing the mucolytic agent N-acetyl cysteine at a concentration of 1% (w/v). After 30 min the stomachs are removed and thoroughly rinsed in fresh Krebs. The tissues are then reincubated under the same conditions in Krebs buffer containing the test compound under investigation. The incubation is terminated after 2.5h by transfering the tissue to 0.25M ice-cold sucrose. After 5 min the stomachs are stained by immersion for 90 min in 10 mls of alcian blue solution (0.1% in 50 mM sodium acetate pH 5.8 containing 0.15M sucrose). Excess dye is removed by washing the tissue for 2 x 15 min periods in 10 ml of 0.25M sucrose.

The alcian blue stained mucus is destained by soaking for 2h, with periodic shaking, in 15 mls of 0.5M magnesium chloride. The blue supernatant is decanted, clarified by shaking with diethylether, and the optical density of the aqueous layer read against a buffer blank at 600 nm.

We have been able to demonstrate significant changes in alcian blue binding by stomachs exposed to carbenoxolone and to 16,16 dimethyl PGE_2 when compared to controls (Table 1).

Table 1 The effect of compounds on mucus synthesis

Sample		Optical Density x 100 (Mean ± s.d., n=6)	
Control		29.0 ± 4.2	
16,16 DiMe PGE_2	1.0 μM	32.2 ± 5.1	$P > 0.05$
16,16 DiMe PGE_2	2.5 μM	40.8 ± 5.0	$P < 0.1$
16,16 DiMe PGE_2	6.3 μM	42.2 ± 3.4	$P < 0.001$
Control		36.8 ± 8.1	
Carbenoxolone	0.1 mM	28.5 ± 2.3	$P < 0.05$
Carbenoxolone	1.0 mM	57.8 ± 7.4	$P < 0.01$
Carbenoxolone	10.0 mM	59.0 ± 7.7	$P < 0.001$

Corne, S.J., Morrissey, S.M. & Woods, R.J. (1974). J. Physiol. (London), 242, 116p-117p.

'BY HAND' CURVE FITTING PROGRAM FOR THE ANALYSIS OF MULTIPLE SITE RADIOLIGAND BINDING DATA

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In radioligand binding studies many ligands interact with multiple receptors (or binding sites) concurrently, and the subsequent binding curves display complex dose/'response' relationships. A variety of analytical methods have been developed to examine data of this form with varying degrees of analytical and statistical sophistication. Dissection of components using graphical methods relying on Scatchard plots (Rosenthal, 1967) lie at one extreme, whilst highly sophisticated curve fitting algorithms (eg LIGAND) lie at the other (Munson & Rodbard, 1980). We present here a simple computer program using the high resolution graphics facility of the Apple microcomputer that bridges these extremes, providing a robust and simple user controlled analytical method, together with some of the statistical methods more usually associated with curve fitting methodology.

Data is input via the keyboard and then displayed in semi-logarithmic co-ordinates for visual examination. The user decides on the number of sites, and enters the values of the parameters (Kd's, Bmax's etc) associated with the chosen model. This curve is drawn and the associated statistics calculated for this curve as a representation of the data. The user is invited to repeat the process to improve upon the initial result, and this cycle is repeated until a satisfactory answer is found. A summary of the session is then produced for final examination.

This program functions as a 'by hand' curve fitting algorithm combining stable numerical munipulations with more advanced statistics. In a laboratory without access to curve fitting routines this program can serve as an improved and easy-to-use method for analysing multiple site binding data, whilst when more sophisticated analytical methods are available, it can be used to find initial values to be used in the main analysis. The ease of constructing complex binding curves easily also makes the program useful as an aid in the design of experimental protocols for these studies.

Although primarily developed for use with radioligand binding data, the program can also be used to examine other dose/response curves, and with modification, could be adapted to model other systems of pharmacological interest.

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COMPUTER SYSTEM FOR CARDIOVASCULAR PHARMACOLOGY

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A computer system has been developed to automate many of the routine functions performed in the cardiovascular pharmacology laboratory. The principal elements of the system are:

1. A laboratory minicomputer with visual display unit. The computer controls the data capture, performs the analysis of the experimental data and generates printed output.
2. A high-performance laboratory interface containing modules for data acquisition, display, timing and control.
3. A 42MByte Winchester non-removable disc for storage of experimental data and programmes.
4. A floppy disc unit for low-cost storage of reduced data and results. The floppy disc also serves as a means of loading programmes on to the Winchester disc.
5. A report-quality pen plotter, equipped with paper advance and shear.
6. A daisy-wheel printer capable of reproducing tables and appendices for direct insertion into reports.
7. A suite of programmes for on-line data capture, analysis and display, and for off-line printing, plotting and manual entry of data.

The computer hardware used in this application is the CED System 3, containing a 16-bit LSI-2/20 processor, 64KByte memory and interfaces to the various peripheral devices. During on-line operations, a moving display of a selected input channel is maintained and a trend graph of derived information is updated on the visual display unit.

The modular software suite consists of programmes written in assembler for data acquisition and in FORTRAN for processing the acquired data, and is arranged so that additional programme modules can be incorporated if necessary. Present processing modules include programmes to deal with pressures, flows, fast and slow contractions and respiratory data. Digital outputs are also available for the control of external equipment, such as physiological stimulators. The sequence of operations is controlled by user-modifiable tables stored as files on the disc. Once the user has selected a type of experiment from a menu, the linkage between the various programmes is automatic, including the correlation of correct data for presentation in printed tables and plotted output.

Acquisition of data from an experiment is performed either automatically or on demand. If intermittent sampling is selected, data acquisition occurs at regular intervals for long enough to ensure a reasonable representation of the signals defined as inputs. For example, a blood pressure signal is searched for evidence of low-frequency respiratory information before the extraction of salient pressure values. Control samples may be requested prior to drug intervention, and continuous sampling takes place for up to 10 minutes after the drug. The data is analysed to determine the times and values of the maximum and minimum excursions of all the data and cross-products of derived values.

INNOVATION AS ASSESSED BY NEW CHEMICAL ENTITIES MARKETED IN THE UK BETWEEN 1960 AND 1982

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The ability to measure pharmaceutical innovation is of importance in an understanding of drug development and the factors that influence this process. Several indices of innovation have been suggested[1,2], but the most valid ones relate either to New Chemical Entities (NCE's) tested in man or reaching the marketplace since it is at these stages that a compound is considered a potential therapeutic candidate. A major study of all NCE's evaluated in man by the UK pharmaceutical industry since the early 60's has been initiated and this paper analyses trends in those marketed in the UK.

A comprehensive list of all new products marketed in the UK and the dates of their introduction was produced from the Monthly Index of Medical Specialities (MIMS) for the period 1960 - 1982. NCE's, defined as new chemical or biological compounds not previously marketed in the UK and excluding new salts or esters conferring no additional therapeutic advantage, were derived from this list and subsequently confirmed as NCE's by the companies originating or marketing the product. Further verification and additional data were gathered from manual and computer literature searches using all available pharmaceutical texts and by comparison with other surveys covering part of the time period for the present study.

The final databank includes details on the generic name, therapeutic and pharmacological categories, brand name, UK marketing date and present availability, and the companies originating or marketing the product. In addition details of patent filing and expiry dates were recorded to enable the effective patent life remaining at the time of marketing and overall drug development times to be calculated.

In the period 1960 - 1963 an average of over 50 NCE's were marketed per year with a sharp decline to around 25 products per annum in 1964 - 1967 and approximately 20 compounds per annum in the remaining period. A total of 616 compounds have been introduced in the 23 years, an average of 27 per year, with one third of these being introduced in the initial four years of the period under study. The NCE's introduced covered every major therapeutic category; 27% were CNS drugs while 20% were antiinfectives and 16% cardiovascular agents. Over half of the drugs originated in Europe with the UK contributing 14%, West Germany 11% and Switzerland 10%. Thirty-seven per cent of the compounds were developed in the US, but only 2% in Japan.

The marked decline in the number of marketed NCE's in 1964 coincided with the introduction of stricter regulations in the UK and the establishment of the Committee on Safety of Drugs. Further research is required to identify the factors involved in this decline but the effect of increased regulatory requirements and the escalating costs of drug development must be considered to be contributing constraints to successful innovation. However, despite these barriers the research-based UK industry has made a significant contribution to the total number of new drugs available, whereas in contrast Japan and other Eastern countries have had little impact on the UK market to date.

1. Pharmaceuticals Working Party, NEDC (1973)
2. Walker, S R (1982) BIRA Journal 1, 34.