



Recovery of Specific Proteins and Enzymes from Blood and Offal Part 2: Growth Factors

The importance of cell growth factors is well understood, with a long-established market for foetal calf blood already in place.

Animal serum is an important source of growth factors for cell culture. Foetal calf serum (FCS) has traditionally been used to stimulate cell growth in isolated mammalian cell cultures. The stimulatory effect is due to a number of factors including the presence of key proteins such as insulin, albumin and transferrin plus the presence of growth factors. These growth factors are at higher concentration in blood from foetal or newborn animals. Adult plasma is generally not suitable, primarily because of the reduction in the level of the growth factors and also because of the accumulation of gammaglobulins (antibodies) in adult animals.

Foetal Calf Serum

Foetal calf serum is used extensively for growing cell lines in culture and is a particularly important commodity item in the biotechnology industry. Serum contains an enormous spectrum of compounds that seem to operate synergistically. It contains little immunogenic protein and high levels of naturally occurring growth factors, which support the growth of a wide variety of cell lines. Premium quality foetal calf serum contains low levels of haemoglobin, no virus and no endotoxins. However, being from a biological system, there is natural variation in the growth enhancing properties of the material from different animal cohorts.

Because it is a commodity item, the price fluctuates considerably. It is disadvantaged in the market because, in terms of quality, there are difficulties in preventing batch-to-batch variability, and it is difficult to meet the strict specifications for endotoxin and some viruses that customers specify.

Cell Releasate

A potential alternative to foetal calf serum used by the biotechnology industry can be prepared from animal blood cells. By activating blood cells it is possible to obtain a fraction that is highly potent in stimulating a wide range of cells to grow in cell culture medium. This substitute, called cell releasate, is easy to produce, highly potent in relation to effect per dose of protein, and broad spectrum in its effect on cells. The material can be used in place of foetal calf serum or as an adjunct in the make up of cell medium.

Releasate is a mix of natural products. Production of releasate does not require the use of unnatural interventionist therapy. The releasate is recovered by treating cells in a physiologically correct manner. The treatments do not require the administration of any product that could be deemed non-physiological or outside the experience of normal cells in a normal body regime.

Cell releasate may be useful in animal production as well as in biotechnology. Releasate has been proposed as suitable for the stimulation of growth rate in domestic animals and in particular for pigs. It is reported that the addition of plasma, in the form of spray-dried protein powder (SDPP), to the diets of early weaned pigs increases feed intake and growth rates at the expense of soy protein, skim milk, or whey protein. The mechanism for these observed effects on pig performances is not well understood. These effects were observed using porcine plasma on weanlings and were predicted from work carried out on mice.

It is suggested that the stimulatory effect of plasma feed proteins may be improved even further by using releasate in combination with dried plasma; or, alternatively, using a SDPP

equivalent that is prepared using techniques that stimulate the production of growth factors. Data indicates that the plasma does not contain these key factors in significant amounts. Reports indicate that bovine plasma is just as effective as the porcine material and perhaps more so.

Market opportunities for releasate

There are a number of serum-free products that currently compete with foetal calf serum in the cell culture media market. Cell Releasate is the name used to describe this potential new substitute for foetal calf serum. This product can be manufactured on demand, produced to a consistent quality and may be manufactured free of endotoxin and without contamination from viruses.

There are some drawbacks associated with the use of serum-free media (ie without FCS) including slower growth rates; however, there are notable positive features which may outweigh any negative considerations.

- The consistency of the product and the degree of control that the investigator can exert over the properties and composition of the medium are impossible to duplicate with foetal calf serum.
- The reduction in the protein content in the growth medium is also very important as it can simplify the downstream processing steps in the recovery of value-added products.
- Most importantly the biological advantage of using proteins which are certifiably free of viral contamination cannot be overlooked.

The outlook for cell culture media is quite strong with some portions of the market showing signs of maturity if not decline, and emerging opportunities which includes the serum-free or foetal-calf serum-replacement culture market.

The world market for media formulations for animal, microbial, plant and other cell culture was an estimated USD\$737 million in 1987 and growing at around 10%–15% a year. This excludes estimates for media used in large-scale fermentations. In 2000 the annual value can reasonably be expected to have grown to some USD\$2,500 – 4,500 million per annum.

Estimates from sources in the industry vary but most expect the serum-free products to take a significant share of the serum market, as much as 40%. This gives a value to the serum-free market of some USD\$1,000 – 1,800 million.

The most important group of reagents in cell culture has been growth factors. The development of recombinant growth factors has helped, and will continue to help, the development of the serum-free market.

The development of serum-free releasate mimics, to some extent, the response that takes place in foetal blood when it is collected and allowed to clot. Instead of foetal blood cells, the process uses washed adult blood cells that are easy to prepare, are cheap and can be produced on demand. The technology for their recovery is common and several cup centrifuges can process over 200 litres of blood per day.

Releasate works at low protein concentration, can be sterilised more readily than foetal calf serum and can be prepared free of virus and endotoxin. It offers the broad-spectrum advantages of foetal calf serum at a fraction of the protein content and at a lower cost to prepare. It could be targeted for specific cell lines and has a good opportunity to tap into the projected market share for serum-free growth media.

Releasate production

Cell releasate, for use as foetal-calf serum replacement, is produced by adult bovine blood cells when they are subjected to stress. The cells are recovered from animal blood by centrifuging. The cells are washed to remove plasma proteins including IgG. They are challenged with thrombin and calcium ions, incubated and then the extracellular medium is removed by centrifuging. The volume recovered (named the *releasate*) is equivalent to the initial volume of blood. The protein level in the product is less than 5% of that in foetal calf serum. The product can be produced in a facility capable of batch producing crude plasma using identical equipment.

The main capital equipment costs include:

- a suitable facility 200m² = \$300,000
- five 6 x 1 litre cup centrifuges = \$120,000
- vacuum peristaltic pumps = \$5,000
- drainage table and plumbing = \$8,000.

From a plant of this size, releasate suitable for cell culture can be produced for an estimated \$12 a litre at a rate of 700 litres of releasate per 5-shift week.

As for all biological products, access to markets requires compliance to US FDA regulations, the 'Harmonious European Union Code', the Japanese sponsorship approval system or the GMP guidelines in Australia.

There is also a good likelihood that releasate would be used as feed supplement for poultry and pigs. Comparative products are available from the American Peptide Company designed to stimulate feed conversion. This cruder feed supplement material could be produced for less than \$1 a litre.

The releasate technology is suitable for introduction to a meat works where access to fresh blood, possibly categorised on the basis of sex and pregnancy, would be available.

Cell releasate products and applications

Potential products are :

- serum-free cell culture media produced to a consistent quality and free of endotoxin and contamination from viruses;
- a novel bovine-derived growth supplement for the stimulation of feed intake and growth rate in pigs and other animals. The product will compete with other products such as hormone preparations that are designed to

stimulate feed conversion. As the product will probably be synergistic to amino acid supplements, it should therefore not be reviewed as a competitor to hormones in the strict sense;

- novel growth supplements derived from ovine and porcine blood cells for use in feed lots for beef production;
- a growth stimulant to promote wound healing in animals;
- a novel testing procedure for screening the potential stimulatory effects of plasma fractions on animal growth using small animals.

Further reading

This information is a summary of information from the following project funded by the Meat Research Corporation:

- Project UGR.002: Value Added Proteins and Enzymes Recovered from the Meat Industry.

Further detail is available from the final project report of this project which is available from Meat and Livestock Australia.

Related information is given in the MLA Co-products brochure:

- Recovery of specific proteins and enzymes from blood
Part 1 – Aprotinin, transglutaminase, fibronectin and related proteins.

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