

Immunomodulators

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Controlled Release Microspheres for Veterinary Applications

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Controlled release technology offers many obvious advantages in the field of veterinary medicine. Less frequent handling of animals and more efficient drug utilization, possibly resulting in lower tissue residues and better efficacy are two of the major reasons companies and researchers investigate this technology. However, a review of the past two decades reveals that few control release parenterals have been commercialized in the veterinary field. Substantial cost of development and potentially high cost of goods appear to be key reasons for the lack of products. Exceptions are the various steroid growth promoters used in the beef industry. Synovex, Ralgro, Compudose, Steeroid, Finaplex-S, and Revalor are widely used non-degradable sustained release implants. Growers realize a rather remarkable profit-to-cost ratio with these implants.

Biodegradable polymer excipients are currently the basis for many controlled release products in development. For example, Leupron Depot for treatment of prostate cancer and endometriosis has been a very successful human product. Numerous other human products based on biodegradable injectable microspheres are in the development stage and include vaccines, growth hormone, contraceptives, and CNS agents (Lewis, 1990).

The most widely investigated polymers with regard to toxicological and clinical data are the aliphatic polyesters based on lactic and glycolic acids. Features such as biocompatibility, predictability of biodegradation kinetics, ease of fabrication and regulatory approval in commercial suture applications have attracted investigators to lactic/glycolic polymers. These materials are commercially available from a few sources, including Vetisorb and Medisorb from Medisorb Technologies

International L.P., Cincinnati, Ohio. The homopolymers and copolymers of lactic and glycolic acids are synthesized by the ring-opening melt condensation of cyclic dimers, lactide and glycolide. Due to the asymmetrical B carbon of lactic acid, D and L stereo-isomers exist, and the resulting polymer can be either D, L or racemic DL.

Biodegradation of the aliphatic polyesters occurs by bulk erosion. The lactic/glycolic polymer chains are cleaved by hydrolysis to the monomeric acids and are eliminated from the body through the Krebs cycle, primarily as carbon dioxide and in urine. Because the rate of hydrolysis of the polymer chain is dependant only on significant changes in temperature and pH or presence of catalyst, very little difference is observed in rate of degradation at different body sites. The degradation kinetics are fairly well established for the entire family of homopolymers and copolymers.

Figure 1 shows the approximate biodegradation rates for various homo- and copolymers of glycolic and lactic acids. The 50:50 lactide/glycolide copolymer has the fastest degradation rate of the commonly used DL-lactide/glycolide materials. Increasing the lactide content affords progressively longer *in vivo* lifetimes. A review of the biodegradation literature of these polymers is available (Holland *et al.*, 1986).

Microspheres and microcapsules of lactide/glycolide polymers have received the most attention in recent years, but implants and fiber forms have been reported as well. Solvent evaporation, phase separation, and fluidized bed coating techniques have been employed to form the microparticle formulations. Generally, the parenteral products are based on spherical microspheres 10-200 microns in diameter.

These particles can be administered with a conventional syringe and needle (18 to 25 gauge, depending on the exact size spheres utilized). A liquid injection vehicle (1-2cc), usually aqueous based, is used to suspend the microspheres for injection.

Typically, the microsphere formulations comprise many thousands of individual monolithic spheres which release the active agent *in vivo* by a combination of drug leaching and polymer erosion in the presence of water. There is a distinct difference in behavior between water-soluble agents and water-insoluble agents in these systems. Water-soluble drugs, especially the higher molecular weight proteins, are more difficult to formulate. New bioactive agents derived from recombinant DNA and hybridoma techniques are especially challenging in controlled-release formulation development. Fragmentation, oxidation, dimerization, unfolding, aggregation and adsorption are often causes for loss of activity of those proteins. Bovine growth hormone is a prime example of a molecule which has not been successfully formulated in the lactide/glycolide polymers. Nevertheless, many classes of drugs have been successfully formulated in the lactide/glycolide polymers. Examples of some of the promising products in our research and development programs are presented here.

Controlled-Release Steroids

The most successful lactide/glycolide steroid formulations have been those of Beck and co-workers (1980). Norethisterone, levonorgestrel, testosterone, testosterone propionate, progesterone, norgestimate, and ethinyl estradiol have been formulated for human and animal applications. Figure 2 shows a serum profile for a 90-day testosterone formulation designed for hormone replacement in males (Atkins *et al.*, 1992a).

This concept has now been extensively pursued in the animal science field. Biodegradable formulations based on estradiol benzoate, trenbolone acetate, and zeranol have been developed for weight gain enhancement in beef cattle. A single

injection of the biodegradable microspheres provided efficacious levels of drug for periods of 3 to 7 months depending upon formulation design and grower/market requirements. Figures 3-5 represent research formulations of these compounds tested in calves. A key advantage of this approach is the potential for combining two or more active agents, such as estradiol benzoate and trenbolone acetate, in one injection. Each drug can be microencapsulated separately to afford unique and independent release profiles. Further advantages include ease of dose titration (simple adjustment of amount injected) and capability to achieve programmed release profiles with multiphasic patterns or delayed release. Weight gain and feed efficiency studies in trials of several hundred animals confirmed the remarkable performance of the steroid growth enhancers delivered via polylactide/glycolide excipients.

A progesterone system for estrus synchronization in sheep has been investigated. Figure 6 shows a serum progesterone profile for a microsphere formulation based on a 50:50 copolymer of lactide/glycolide.

Controlled Release Vaccines

Vaccine delivery systems designed to provide an effective single-injection immunization and eliminate the need for booster injections are highly sought in the human and veterinary fields (Hazrati *et al.*, 1992). The availability of controlled-release systems with biodegradable polymers has brought this goal within reach. A variety of antigens including bacteria, toxoids, enveloped and naked viruses, recombinant proteins and conjugated proteins have been microencapsulated in polylactide/glycolide. These vaccines offer potential for administration via injection, oral, and reproductive tract routes.

Due to the relatively high molecular weight and size of the antigens, biodegradable excipients which deliver the agent by erosion are proving very useful in this area. Further, microsphere formulations offer the potential of

programming the antigen delivery. Figure 7 is a schematic depicting a complex formulation with independent components (represented as A - D) each designed to release antigen at a different time. The composite antibody response is shown as well. Thus, a product could be comprised of only one component or several components. The release of antigens from the biodegradable microspheres is determined primarily by the antigen:polymer ratio, the microsphere diameter, and the polymer biodegradation kinetics. The polymer kinetics are controlled by the lactide:glycolide ratio in the copolymer and the initial molecular weight. These key parameters can be manipulated to afford the desired immune response.

Recent studies have shown that chickens immunized with *Salmonella enteritidis* - containing microspheres produced continuous antibody levels comparable to those of chickens given multiple injections of antigen over a period of more than 100 days (Figure 8). Current research is aimed at achieving higher antibody levels for longer periods of time (Hazrati *et al.*, 1993).

Contraceptive vaccines for humans and animals also are being investigated. The human vaccine consists of a synthetic peptide representing a hCG specific region of the C-terminus of the hormone beta subunit. The antigen is coupled to diphtheria toxoid (bHCG:dt). Figure 9 shows the antibody response in rabbits resulting from a polylactide microsphere formulation of this antigen. A single injection afforded elevated levels over one year. A repeat injection sustained the antibody production for another year (Stevens *et al.*, 1992).

An animal contraceptive based on a polypeptide (LHRH) conjugated to diphtheria toxoid has also been studied. Figure 10 shows the antibody response from a single injection with a polylactide microsphere product. This product is under development for use in companion animals (Lewis, 1990).

Other Application

Many other categories of active agents in combination with lactide/glycolide polymers are presently being investigated. These products are aimed at both food-producing and companion animals.

For example, a melatonin delivery system has received attention both in production of wool and fur and in reproduction manipulation. Figure 11 represents the serum melatonin levels in goats treated with a lactide/glycolide microsphere product and the fact that changing the dose results in a change in serum levels. A single injection afforded levels over several months. A similar product shortened the fur production time in mink by more than six weeks.

Narcotic antagonists such as naltrexone have rather unique applications in the animal health field. These compounds have proven useful in preventing undesirable behavioral characteristics such as tail chasing in dogs and crib biting in horses. A controlled-release naltrexone formulation based on lactide/glycolide polymers has been tested. Pharmacokinetic studies in baboons confirmed that a 30-day release profile is feasible from a single injection (Figure 12). Products to treat narcotic addiction in humans are being developed (Atkins *et al.*, 1992b).

Conclusions

The feasibility of controlled drug delivery from biodegradable microspheres is well established. Several products based on this approach are fully developed and a few are commercially available. Single injections can afford efficacious levels of drug for periods of several days to a year or more. These unique formulations offer important advantages in programming the system to achieve desired goals in human health care and veterinary medicine.

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Figures

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- Figure 2. Serum testosterone levels in human clinical trial
- Figure 3. Trenbolone levels in calves
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- Figure 6. Progesterone levels in sheep
- Figure 7. Schematic of programmed vaccine delivery from microspheres
- Figure 8. Antibody levels in chickens immunized with two microsphere formulations
- Figure 9. Antibody levels in rabbits immunized with bHCG:dt conjugate in microspheres
- Figure 10. Antibody response to LHRH conjugate in microspheres
- Figure 11. Melatonin levels in goats
- Figure 12. Naltrexone levels in baboons

Figure 1.

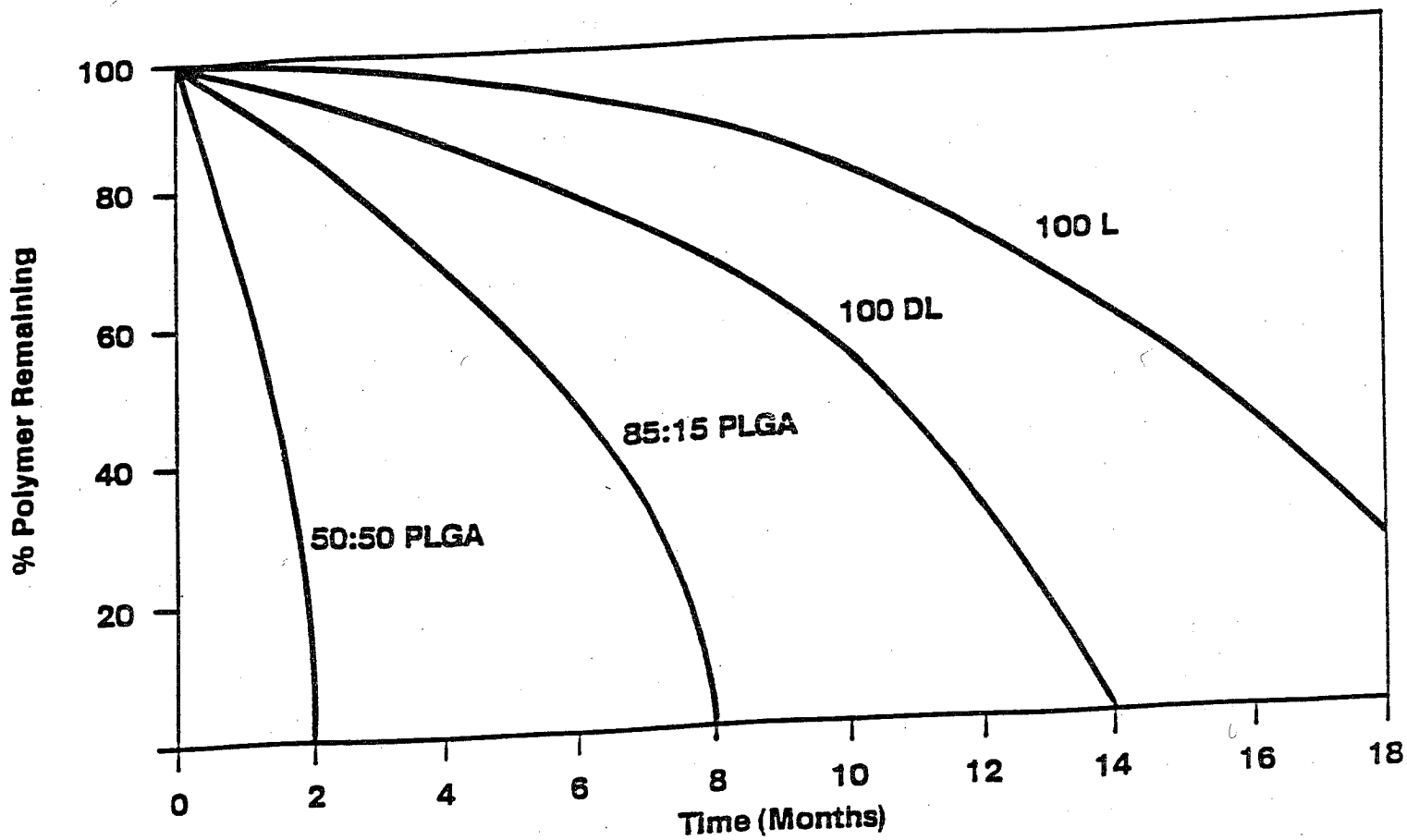


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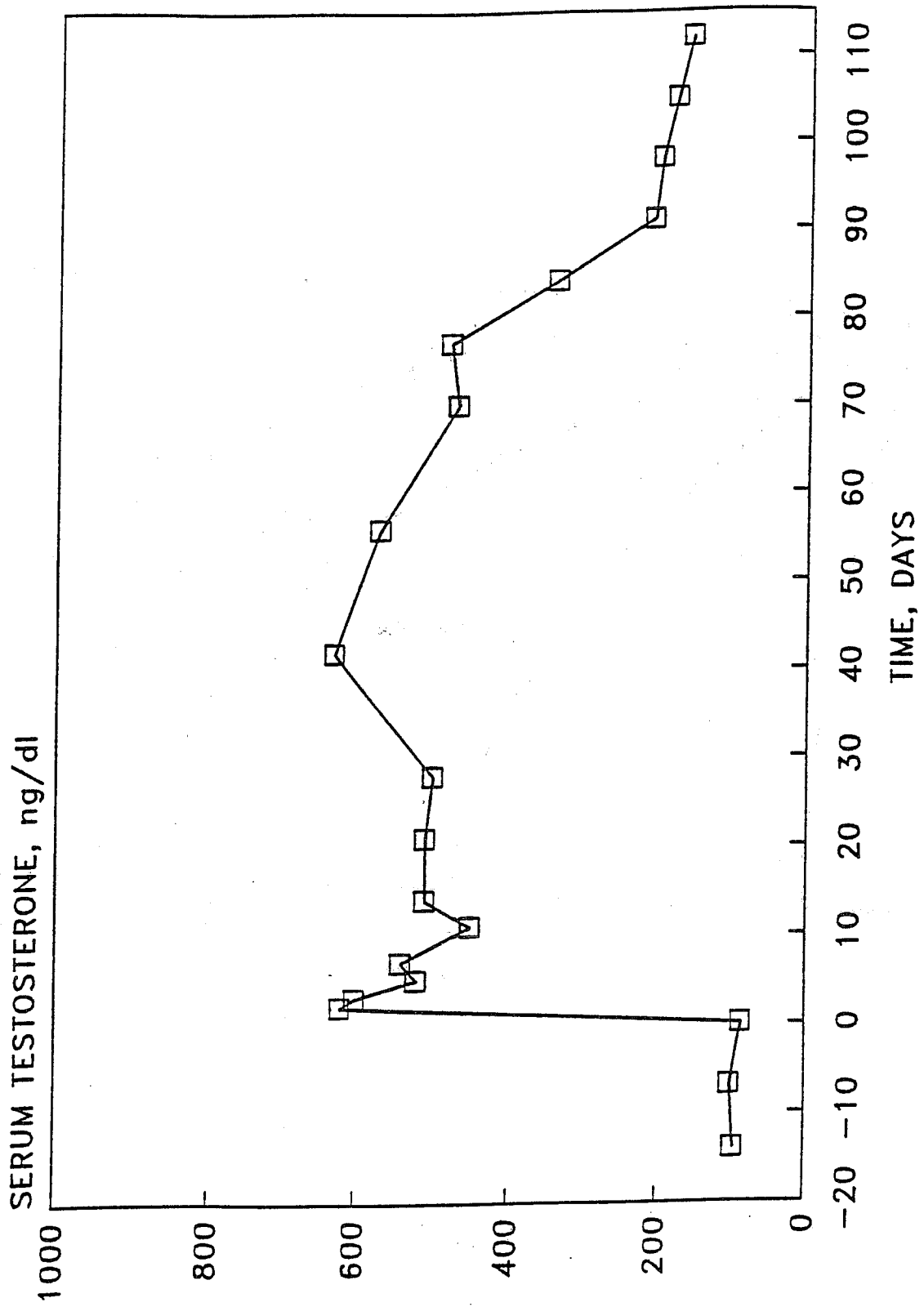


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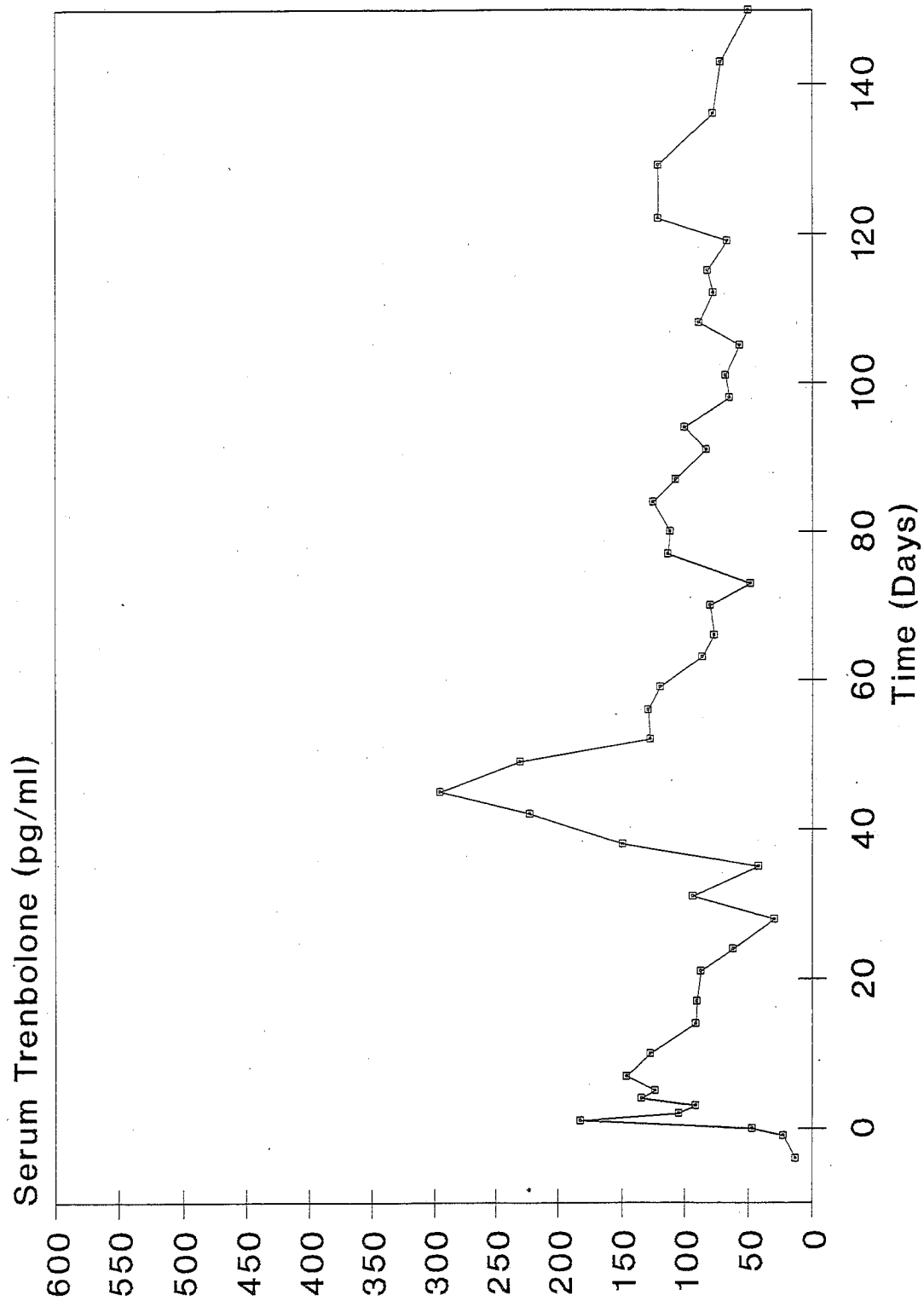


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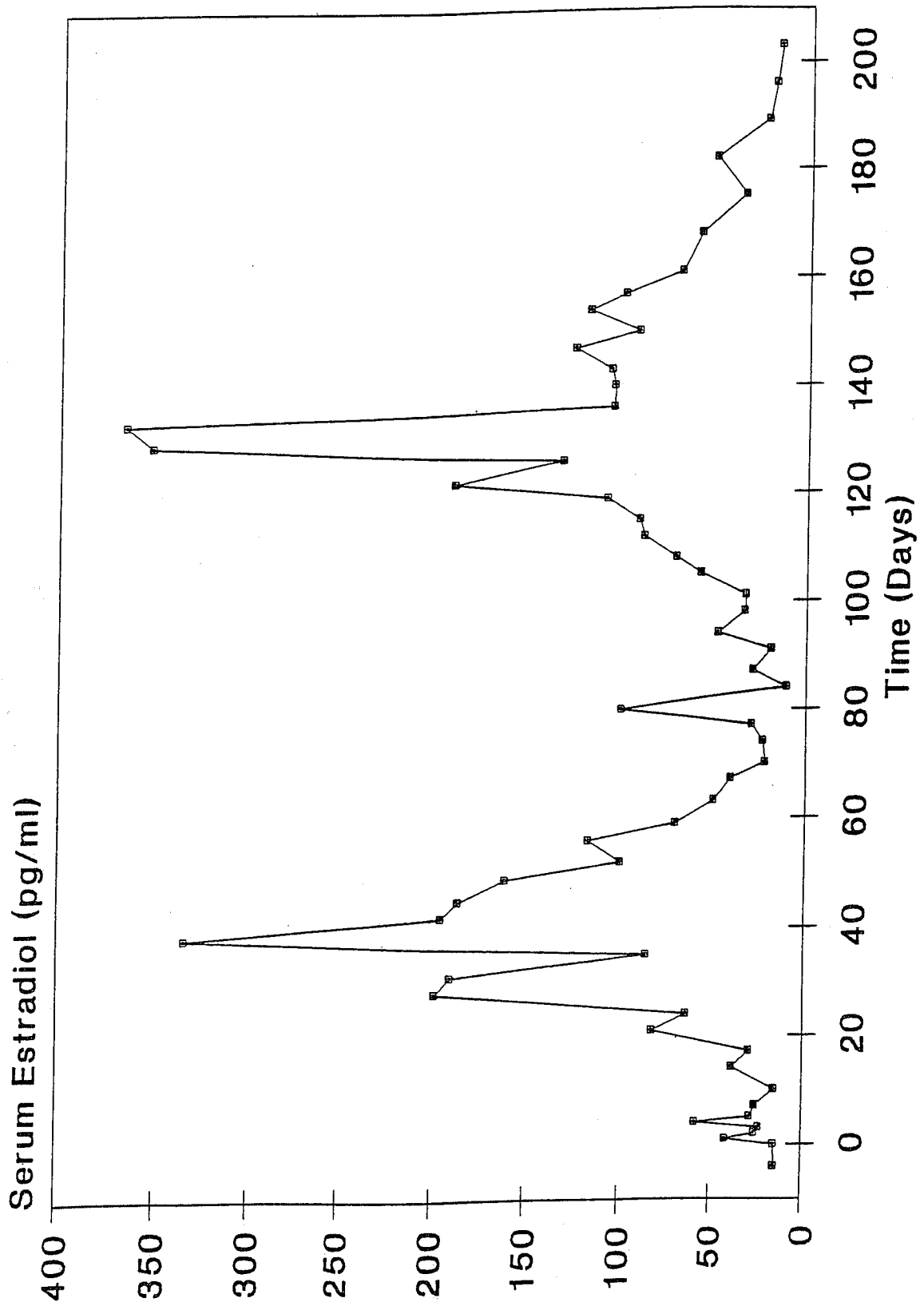


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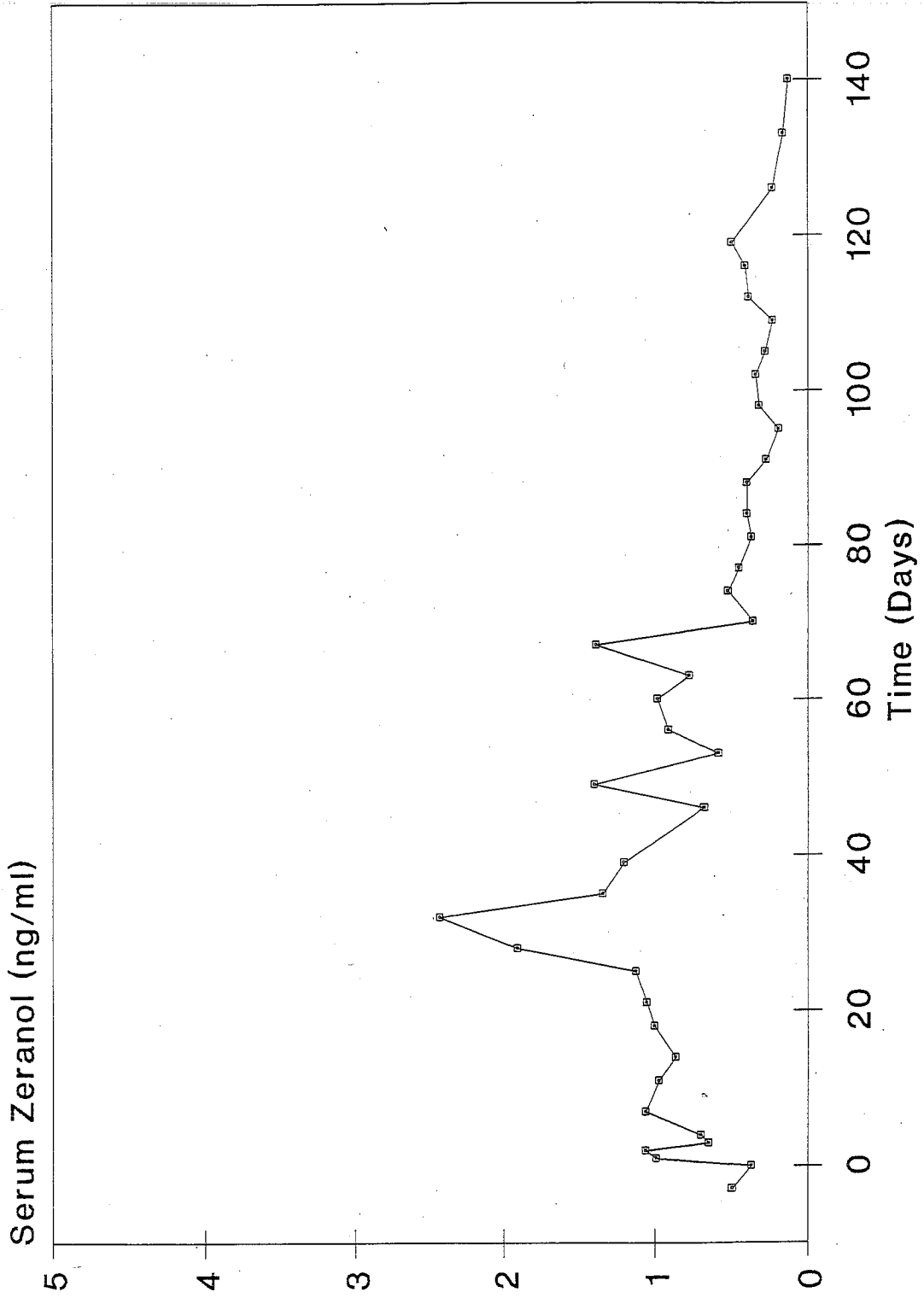


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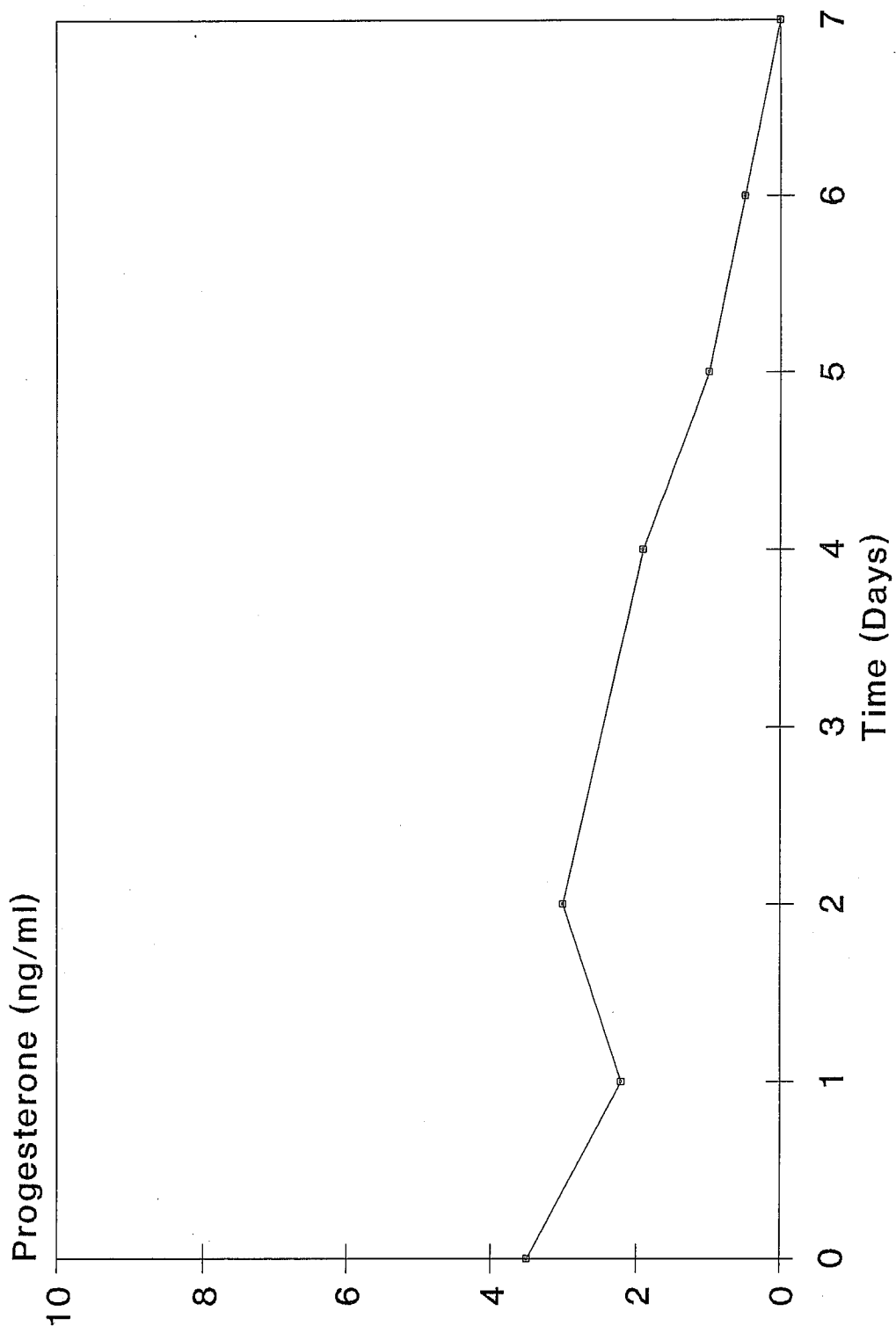


Figure 7.

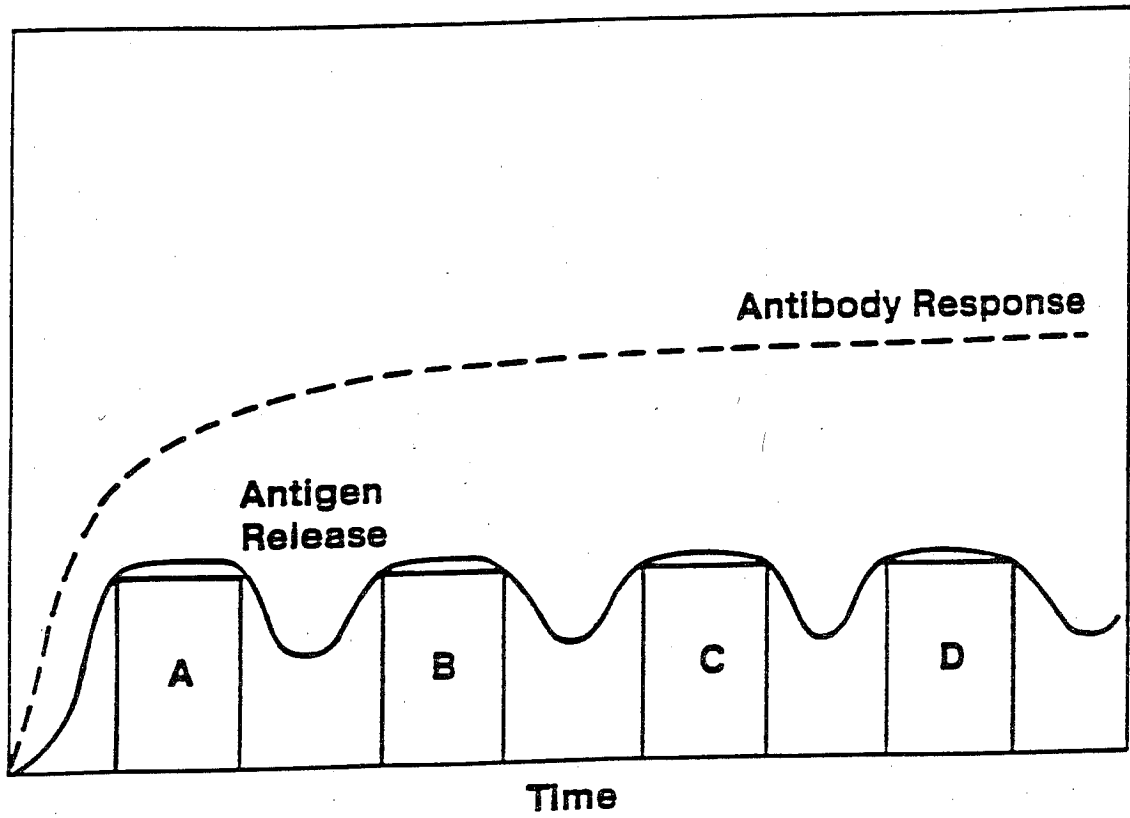


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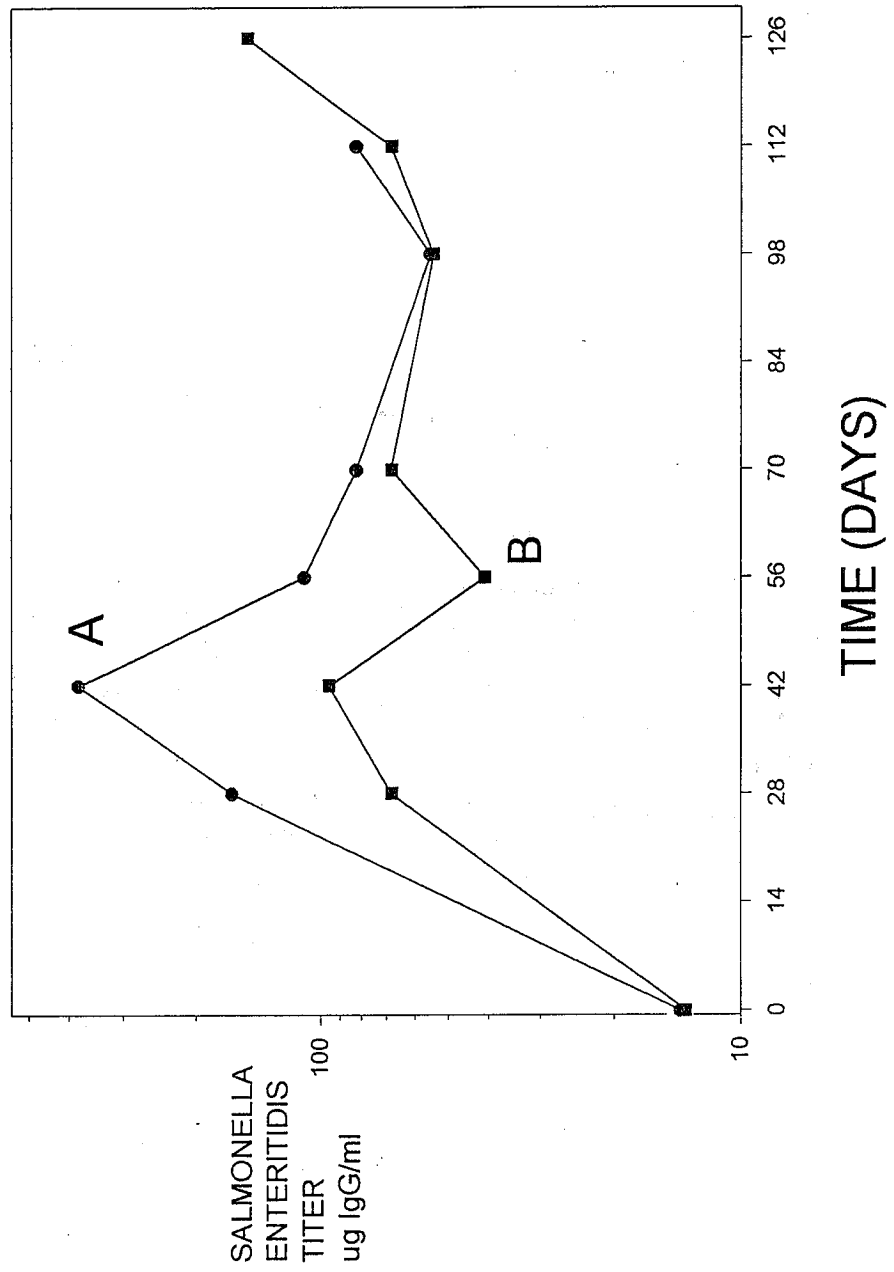


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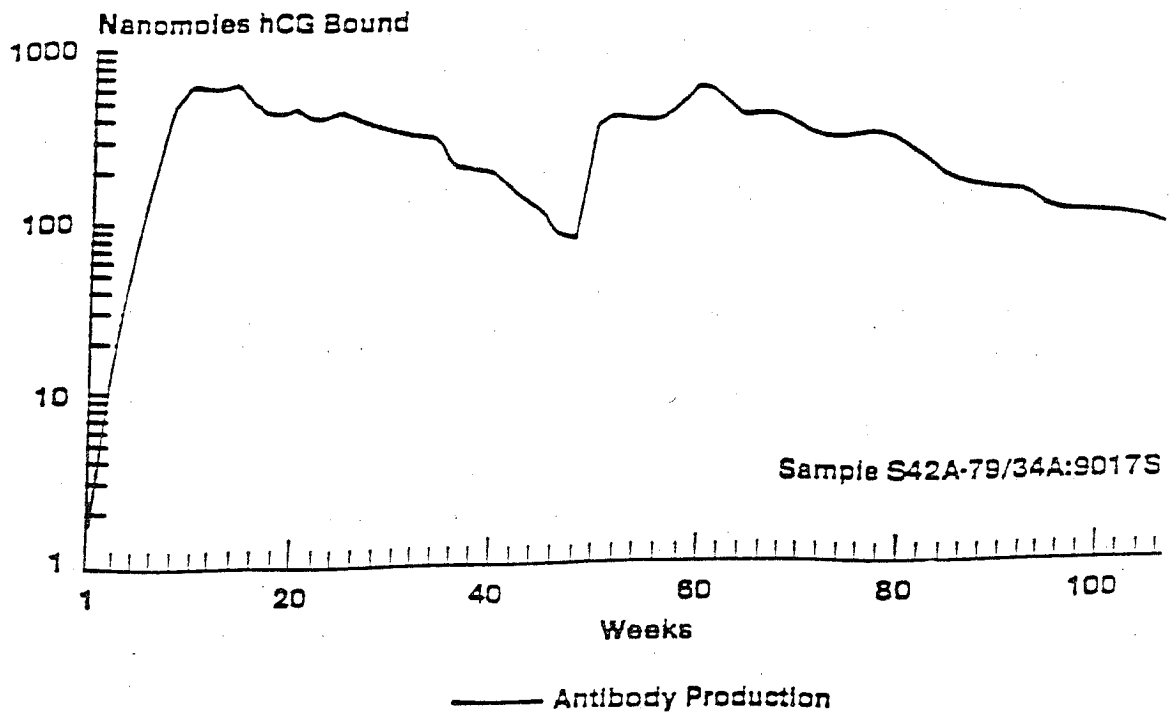


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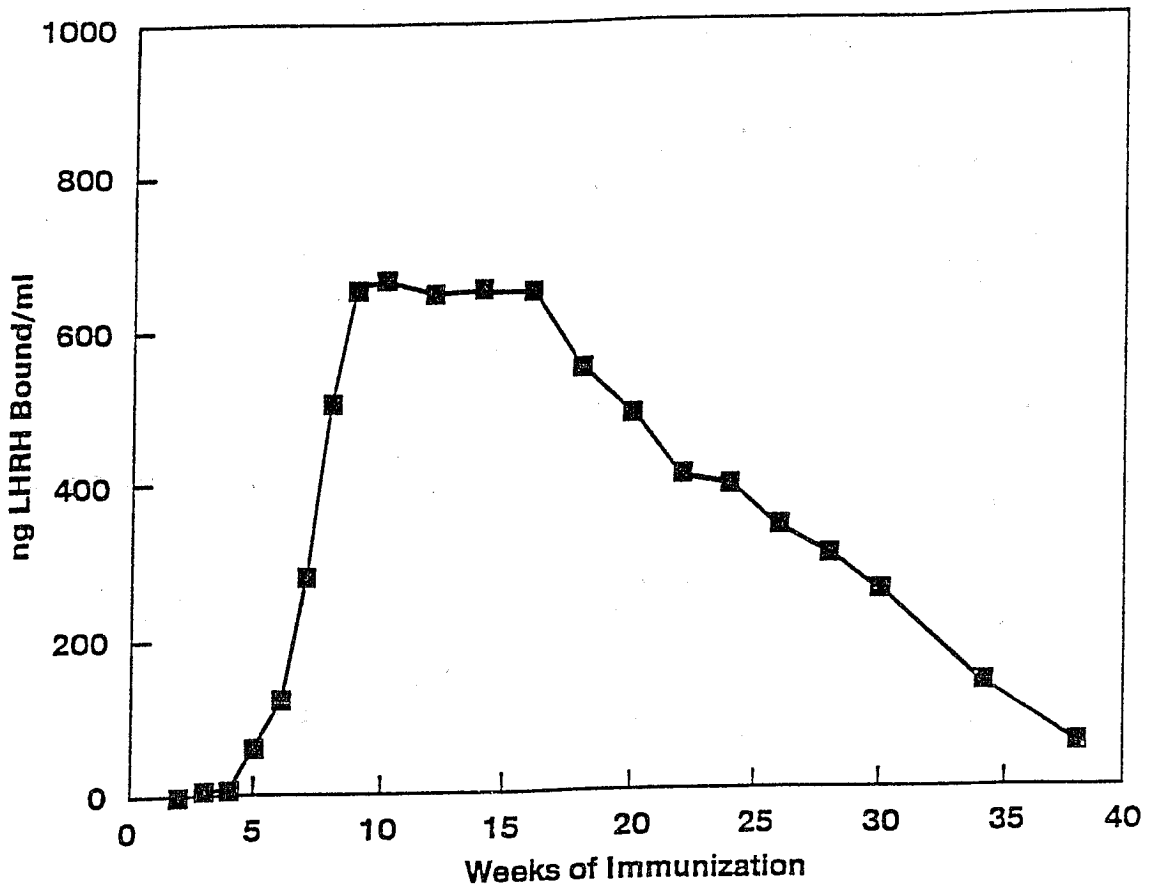


Figure 11.

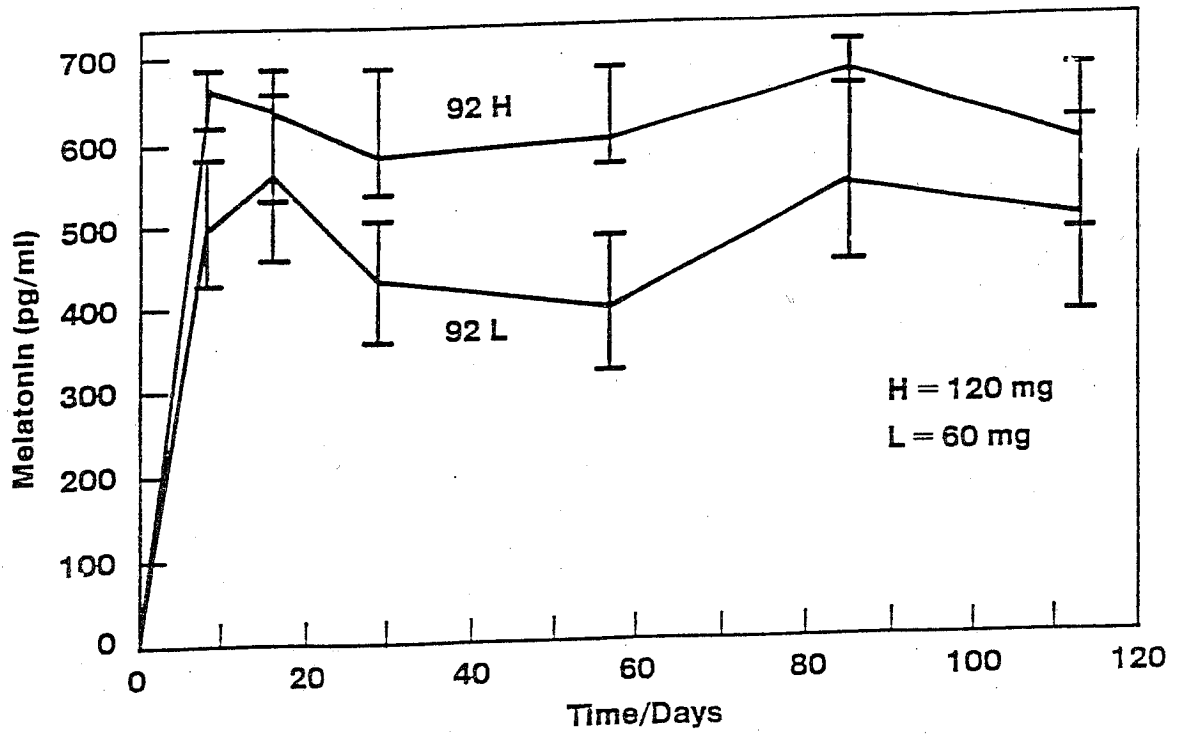


Figure 12.

