Hemoperfusive Removal of Specific Intoxicants: The Role of the Rabbit in Preclinical Trials

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Introduction

Our hope is for the ultimate replacement of laboratory animals by chemical, bacterial or tissue culture technology. However, at present and in the forseeable future, small animals are absolutely necessary for trials of new drugs and surgical and other invasive procedures before clinical trials in humans.

The extracorporeal techniques: continuous flow plasma/cell separation (blood banking, oncology) and hemodialysis and hemoperfusion (nephrology and emergency medicine) are commonplace in clinical medicine. Hemoperfusion is becoming the method of choice for direct detoxification of whole blood and is being used with increasing frequency for routine as well as for unusual therapies. The major adsorbents commercially available, charcoal and exchanger resins, are nonspecific. They complex useful biomolecules in addition to the toxic compounds. There may be little choice but to use them in emergency situations; but this points to the need for the development of specific adsorbents. Obviously these must be validated in laboratory animals before human trials.

Research in our laboratory is progressing along several major lines including the design and development of specific adsorbents (a) to remove specific antibodies and/or immune complexes (auto immune syndromes), (b) to remove undesirable molecules, inadvertantly (iatrogenic) or purposefully (suicide attempt) ingested or 'idiopathically' elevated (i.e., hypercholesterolemia) and (c) other related uses.

We have developed a novel sorbent system, agarose-polyacrolein microsphere beads (APAMB) (8, 9). These are monodisperse agarose beads of 0.5–1.0 mm diameter containing thousands of microspheres of 0.2 μ mean diameter, encapsulated in an agarose matrix. A specific antigen is covalently linked to the polycrolein microspheres to remove a specific antibody or vice versa. The gel matrix/coating confers physical strength, biocompatibility and special configuration as well as porosity to permit rapid entry of antibodies for reaction.

Thus far we have developed and will soon begin clinical trials of a sorbent for removal of digoxin from digoxin intoxicated patients (11). Already completed animal studies show the efficacy of our newly developed sorbents for the removal of specific antibodies (7), paraquat (1), iron salts (3) and mercury (10) from the blood of intoxicated animals. We have also developed immunospheres with specific antibodies on their surface for labelling and separating T from B lymphocytes (12, 15). These will be useful to circumvent some problems prior to bone marrow transplantation. In addition, magnetic properties can be conferred on the microspheres so that cells can be labelled and then separated in a magnetic field (13). In various stages of design and development are sorbents to remove cholesterol, bilirubin and immune complexes from the blood.

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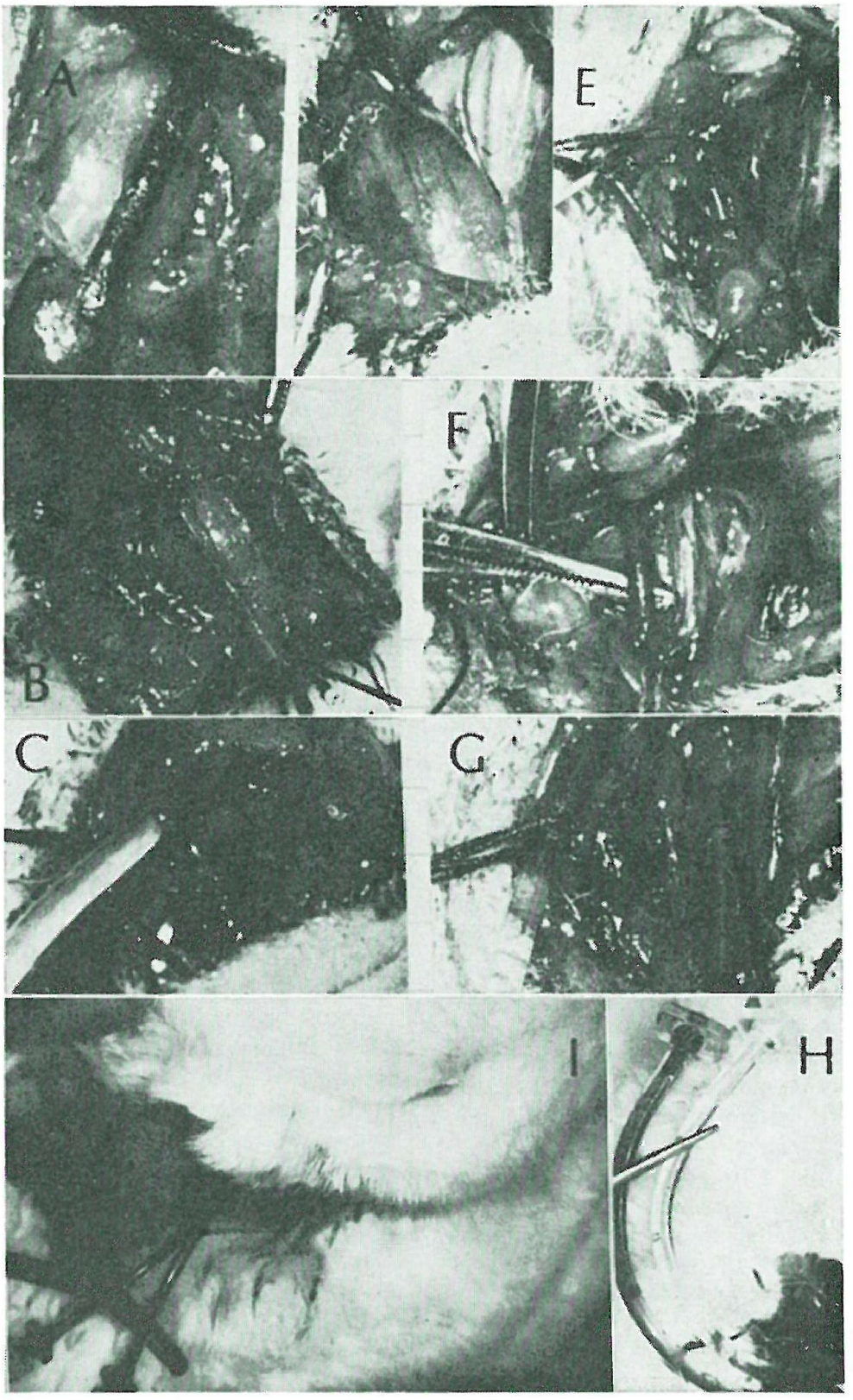

Figure 1. Photographs of the surgical procedure. See text for description.

The rabbit is one of the classical experimental animals used in the basic sciences. Its attributes: convenient size, ease of management, wealth of anatomical, biochemical and physiological data, etc., are well documented (5, 6, 16). Rabbits are being used with increasing frequency for testing medical procedures and surgery (2, 14). Galloway and Lui (4) detailed their use in hemoperfusion with charcoal. In this paper we describe our surgical approach and some of the areas in which we have employed the prepared rabbits.

Surgical approach and results

Animals. Inbred white rabbits (3.5 to 4.5 kg) were obtained from the Dept of Exptl Animals of the Weizmann Inst of Science. In preliminary surgery we used the femoral arteries and veins for blood access. Although this approach is straightforward we found this procedure unsatisfactory because the cannulas were too easily tampered with by the rabbits and the site was too readily soiled. More important, the rabbit lacks a profunda femoral artery (2). Thus not infrequently, circulation to the hind limbs becomes compromised causing complications irrelevant to the hemoperfusion, but serious enough to confound the results of any long term therapy.

Surgical preparation. Ketamine HCl (15 mg/kg) was injected IM; minimal additional ether was required during surgical preparation. Oxygen was given via a face mask prn.

The rabbit is placed supine on a board for full extension of the neck. Surgery begins with a longitudinal paramedial incision in the neck at the medial border of the sternomastoid muscle. The skin is retracted, the external jugular veins are identified, a jugular vein is dissected free of its surrounding tissue (Fig. 1A) and its body end ligated (Fig. 1B). The cannula shown at the top of Fig. 2A (or a suitable length of a French 8 umbilical catheter) is inserted through an oblique incision, propagated 15 to 20 mm in the vein (Fig. 1C) and double ligated with 4–0 silk. The cannulated vein and fat pad are retracted medially. The anterior scalenus muscle beneath the fat pad is retracted laterally. Blunt dissection of its lateral border exposes the carotid sheath (Fig. 1D). The vagus nerve is separated from the carotid artery by blunt dissection (Fig. 1E). The artery is elevated (Fig. 1F) and its cephalic side is ligated with 4–0 silk. After clamping the caudal side, the artery is cannulated 15 mm toward the aortic arch through an oblique incision (Fig. 1G). The cannulated artery and vein are fixated with 2–0 silk by surrounding them with the sternomastoid and scalenus anterior muscles. The rabbit released from the board is positioned on its left side, the free ends of the cannulas are passed through a subcutaneous tunnel and brought out of the skin through an incision between the bases of the ears (Figs. 1H, 1I).

A shunt may be inserted into the tips of the cannulas as desired. For intermittent hemoperfusion during several days, the cannulas are covered by the ears which in turn are taped together at their distal ends to hinder tampering of the cannulas by the rabbit.

Hemoperfusion system. The in situ cannulas are connected to the arterial outlet set (Fig. 2A) via a 3-way stopcock. Arterial blood flows from the carotid artery through the pump segment in the peristaltic pump to perfuse the gel beads in the column. The other side of the column is attached to the venous inflow set which contains a bubble trap and ends in a 3-way stopcock (Fig. 2B). Blood returns via the stopcock to the cannula in the jugular vein. The perspex column (Fig. 3) is a cylinder, diameter and length according to need with standard outer threading at both ends. The column screws into identical end pieces at their distal ends, fitted with teflon connectors. The gel beads are retained by 60 mesh stainless stell screening fitted into

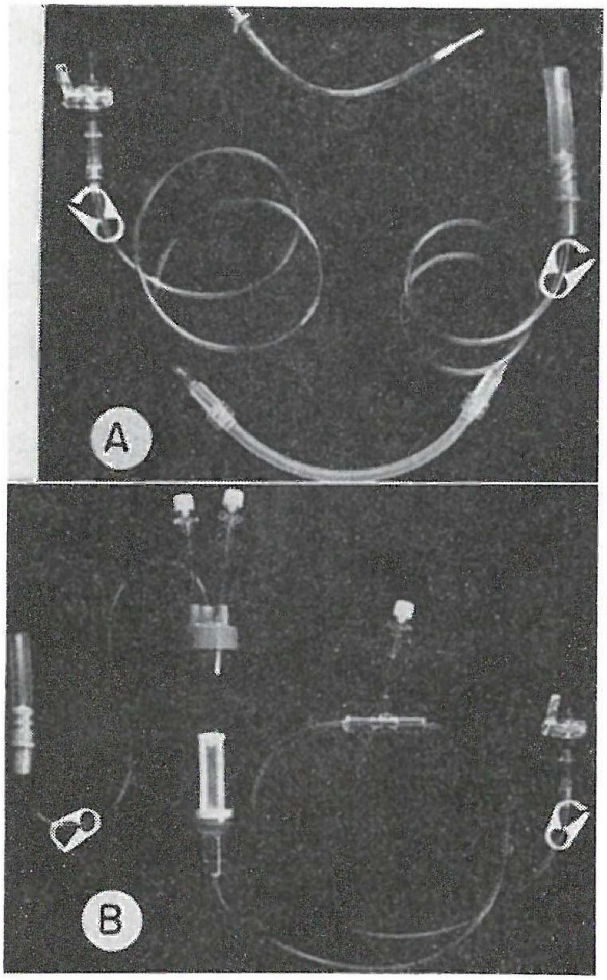

Figure 2. (A) Cannula and arterial outflow set. 3-way stopcock connects to cannula inserted in artery. Length of pump segment according to the blood pump used. Distal end connects to column. (B) Venous inflow set. Connector from column to bubble trap terminates in 3-way stopcockk to return hemoperfused blood to vein. In line Tee connector for infusion, if needed.

the teflon holder. All plastic components are medical grade-biocompatible, sterilized with ethylene oxide prior to use.

A photograph of the hemoperfusion procedure is shown in Fig. 4.

The complete system including tubing, gel beads, bubble trap, etc., were equilibrated with sterile heparinized saline (3 U/ml) and flushed with heparinized saline (1 U/ml) before use. The extracorporeal blood was temperature controlled in a water bath.

Rabbits were fully heparinized by IV injection of 300 U heparin per kg and maintained anti coagulated by discrete addition prn.

Whole blood perfused the APAMB-BSA at speeds of 8 to 15 ml per min so that the entire blood volume perfused the APAMB every 20 to 30 min. Lower speeds were used at times.

Rabbits were lightly restrained though unanesthetized during hemoperfusion. They seemed quite content and usually dozed during the procedure.

The rate of removal of mercury in a rabbit hemoperfusion trial is shown in Fig. 5. In this case 75 % of the mercury was removed in 2 hrs.

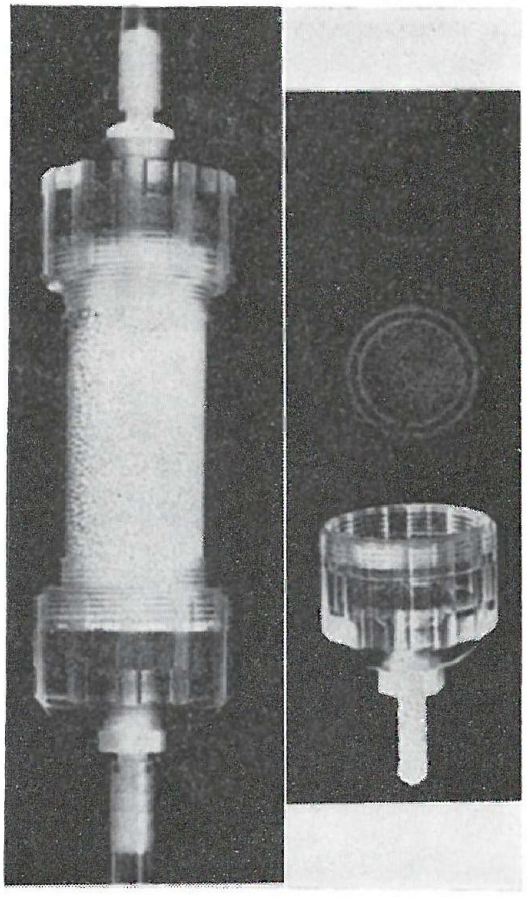

Figure 3. Perspex column to house the gel beads. Tube, dimensions as required, screws into end pieces. Beads retained by 60 mesh stainless steel mesh fitted into the teflon holders.

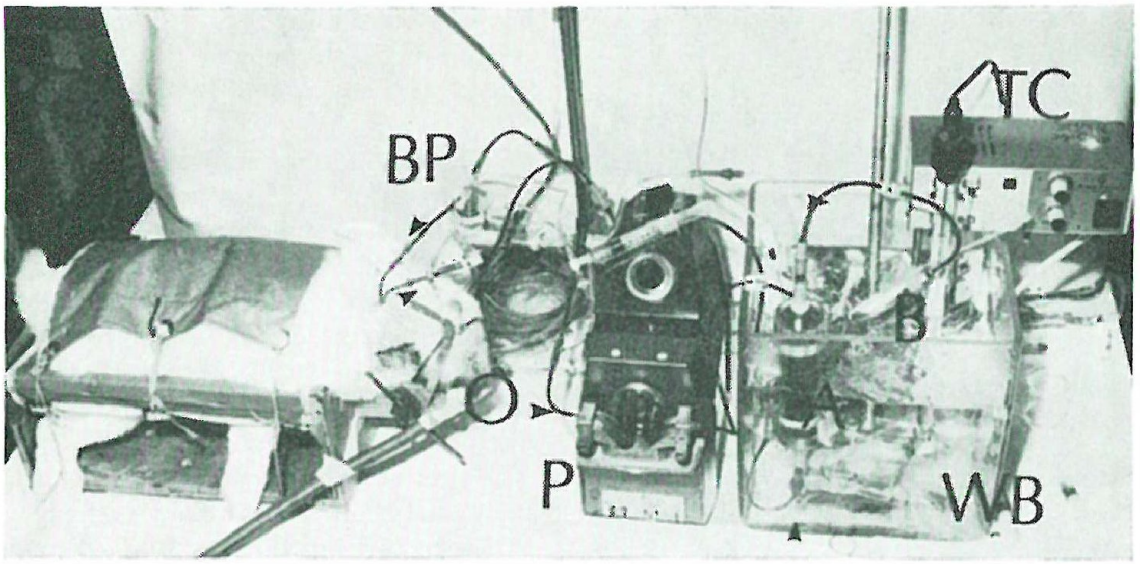

Figure 4. Photograph of hemoperfusion procedure. < = direction of blood flow, P = blood peristaltic pump, A = column, B = bubble trap, V = venous return, TC = temperature controller and water circulation, WB = water bath and BP = blood pressur transducer.

The rate of removal of a specific antibody (rabbit anti bovine serum antibody) is shown in Fig. 6. Over 94 % of the anti BSA was removed from the bloodstream in 89 min and over 99 % by the end of the 3 hrs hemoperfusion.

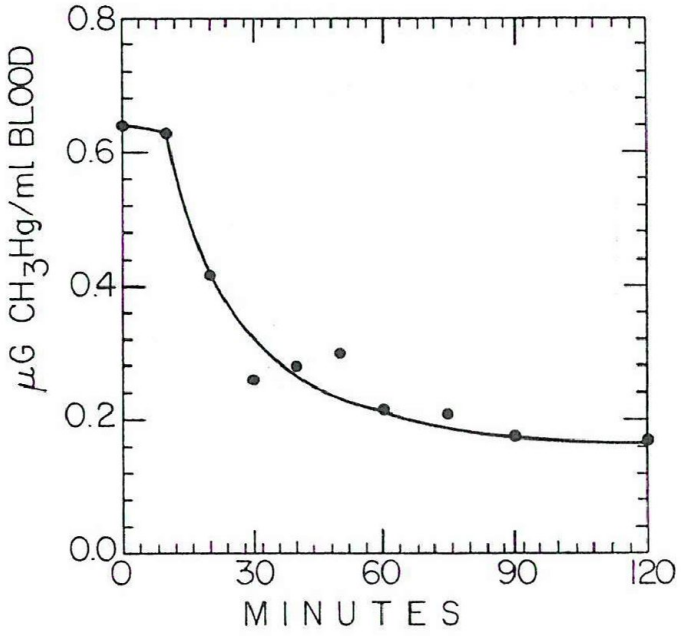

Figure 5. Rate of removal of mercury from dosed rabbit. Arterial blood was assayed. Column contained 15 gm gel beads covalently bound to thio erythritol. Pump flow rate was 10 ml/min.

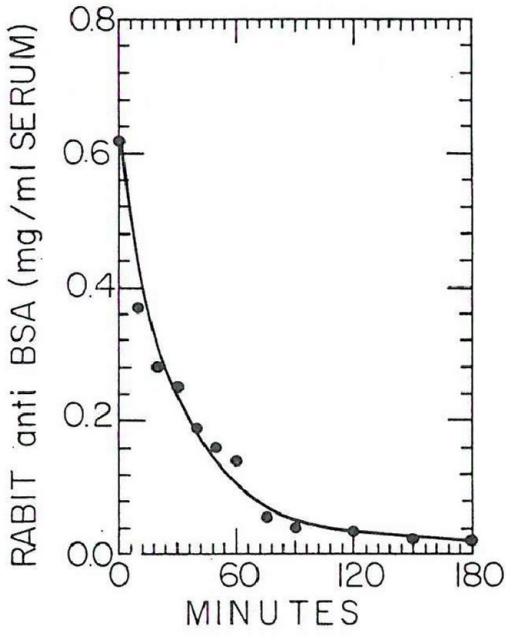

Figure 6. Rate of removal of anti BSA from immunized rabbit. Arterial blood was assayed. Column contained 30 gm gel beads covalently bound to BSA. Pump speed was 12 ml/min.

Not only does the rabbit serve admirably for hemoperfusion, there are certain fields for which it may be extremely well suited. Hypercholesterolemia and its sequela, coronary heart disease are a major problem confronting developed nations. The rabbit in a state of hypercholesterolemia, either induced by diet or naturally occurring in homozygous mutants, should aid greatly in the development of hemoperfusion as a palliative treatment.

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Sammendrag (v. *P. Svendsen*)
Fjernelse af specifikke giftstoffer ved hæmoperfusion
Hæmoperfusion er den foretrukne metode til direkte detoksifikation af patienter med akutte forgiftninger. Som adsorbant anvendes sædvanligvis kul. Den nyeste forskning indenfor dette område beskæftiger sig med udvikling af specifikke adsorbanter til fjernelse af specifikke antistoffer, immunkomplekser og giftstoffer.
Der gives en beskrivelse af en dyreeksperimentel model til udvikling af specifik detoksifikation ved anvendelse af hæmoperfusion. Som forsøgsdyr anvendes kaniner med permanente katetre i v. jugularis og a. carotis. Hæmoperfusionssystemet består af en peristaltisk pumpe og en søjle med agaroseperler (0.5—1.0 mm i diameter) indeholdende tusinder af mikrosphærer (0.2 μ i diameter) koblet til specifikke antigener.
Det arterielle blod pumpes fra a. carotis gennem søjlen til v. jugularis. Systemet perfunderes med hepariniseret saltvand (1 enh/ml) før brug, og kaninen hepariniseres med 300 enh heparin pr. kg legemsvægt. Perfusionshastigheden er 8—15 ml/min, svarende til en perfusionshastighed på 20—30 min.
I fig. 5 og 6 vises resultaterne af forsøg på fjernelse af kviksølv og anti bovint serum albumin.