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## A Modified Apparatus for Dual, Sterilized, Isolated Perfusion of the Rat Liver

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**Abstract** *The isolated perfused rat liver (IPRL) has proven to be a useful model for the study of physiology and pathology of the liver. For research in nonparenchymal cell (NPC) function that includes measurement of cytokine production (eg, TNF), it is necessary to have a sterilized perfusion system. We have modified the IPRL apparatus so as to be able to perform sterile perfusions of two livers simultaneously. The perfusion apparatus is a recirculating closed system in which the oxygenator is a plastic container separated into two chambers by a fenestrated plastic wall. A disposable macropore filter functions as both a bubble trap and perfusate filter. The sterilization process is done by immersing the various components in Benz-All solution. The tubing is disinfected by irrigation with 10% Clorox followed by 0.9% sodium chloride solution. The perfusate used is filter-sterilized Krebs buffer solution containing 0.5 g Mandol/250 mL perfusate. Not only can two organs be conveniently perfused simultaneously, but the entire system can be reliably sterilized for up to 20 consecutive perfusions. Bile production is higher and more stable with less leakage of intracellular enzymes. Many of the components are disposable and can be altered to suit the needs of a particular experiment.*

**Keywords:** Isolated perfused rat liver (IPRL), dual, sterilized.

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The technique and apparatus used for *ex vivo* perfusion of the isolated rat liver (IPRL) have been greatly improved since they were first used about 100 years ago. IPRL has proven to be a useful model for the study of normal and abnormal physiology of liver.<sup>1-3</sup> In recent years, research in hepatology has expanded to studies of the NPC populations of the liver. Whole bacteria as well as bacterial particles can alter NPC function. At certain concentrations, bacteria and bacterial particles activate NPC. It has therefore become necessary to develop a sterilized IPRL apparatus and perfusate for studies of the intact liver. For example, measurement of the effects of cold and warm ischemia on the Kupffer cells, endothelial cells, and hepatocytes, or the measurement of cytokine production (eg, tumor necrosis factor (TNF), which is produced by the Kupffer cells), requires a sterile perfusion system. Sterilization of the IPRL apparatus and perfusate is time-consuming. However, we have further modified the IPRL apparatus so as to be able to perfuse two livers simultaneously. Therefore, two intact livers can be perfused simultaneously for 2½ to 3 h, each in less time than it would take to do them separately. Liver perfusions can be done with reproducible bile production, leakage of hepatocellular enzymes, and production of tumor necrosis factor.

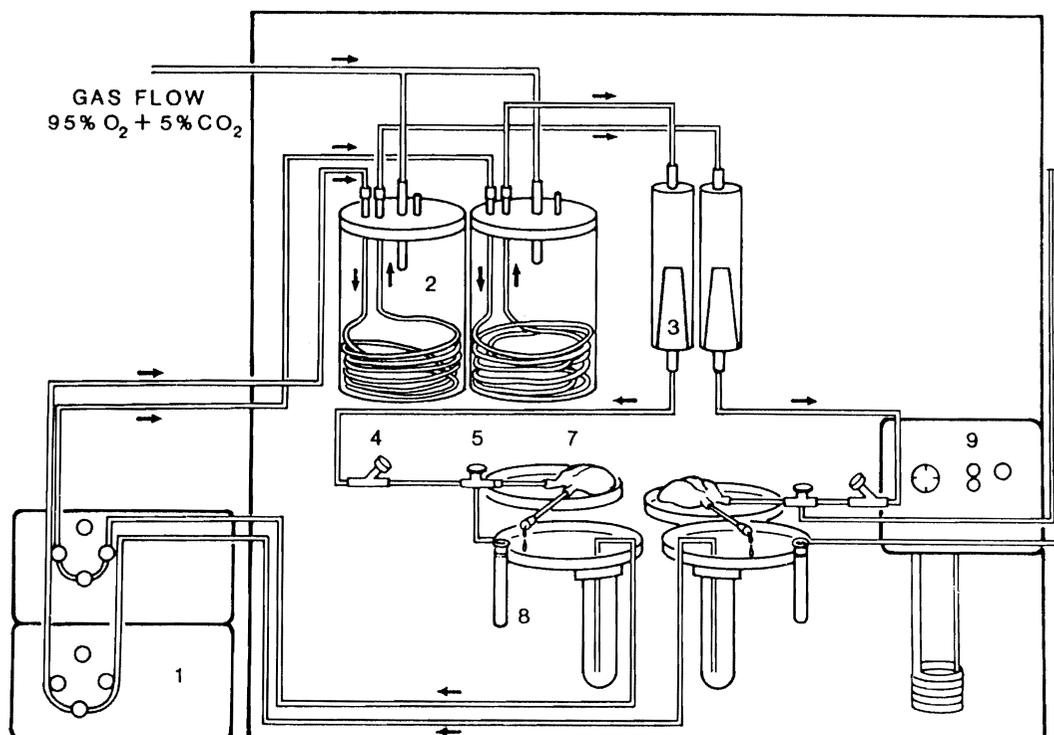
### Material and Methods

Male Lewis rats (250–350 g) were used as liver donors. All animals are induced and maintained with inhalational methoxyflurane. Prior to harvesting the liver, 300 units of heparin are injected via the penile vein. The surgical procedure is similar to that previously reported<sup>4-6</sup> except for some minor modifications. A transverse incision is used to expose the upper abdominal cavity, which allows access to all the supporting ligaments of the liver and to the suprahepatic inferior vena cava (IVC). The portal vein is cannulated with a 16-gauge angiocatheter, while the infrahepatic IVC is cannulated with a 14-gauge catheter. The larger angiocatheter in the IVC allows a decreased perfusion pressure (10–14 cm H<sub>2</sub>O) at high flow rates (2.5–3.5 mL/min g liver<sup>-1</sup>). This improves the delivery of oxygen without barotrauma to the hepatic vasculature as is evidenced by decreased swelling and weight gain of the livers during a perfusion. The suprahepatic IVC is ligated at the level of the diaphragm immediately after cannulation of the infrahepatic IVC. Harvest of the two livers is staggered from 30 to 45 min to allow for adequate spacing of sample collection during the perfusion. The second liver is harvested during the beginning of the first perfusion. Each liver lies on a separate petri dish, which is covered by a second, inverted dish (Fig 2). We routinely allow a 15-min period of equilibration ( $t = 15$  to  $t = 0$ ) after the liver is placed on the perfusion apparatus for the bile production and temperature of the liver to stabilize.

The perfusion apparatus is a recirculating system and has three major parts that are housed within a thermostatically controlled cabinet: a perfusion flow controller, a pump, and an oxygenator.<sup>2,7,8</sup> The following modifications have been made in the apparatus (Fig 1) and the technique of perfusion.

#### *The Oxygenator*

A covered plastic container that is used for the outer housing of the oxygenator has been separated into two chambers by a piece of plastic that has been fenestrated. This allows free flow of oxygen and carbon dioxide between the two chambers. The oxy-



**Figure 1.** Schematic diagram of dual perfusion apparatus: 1, pulsatile pump; 2, oxygenator; 3, filter and debubbler and reservoir; 4, perfusate sample port; 5, three-way stopcock; 6, manometer; 7, liver platform and collecting basin; 8, bile collector; 9, constant temperature circulator.

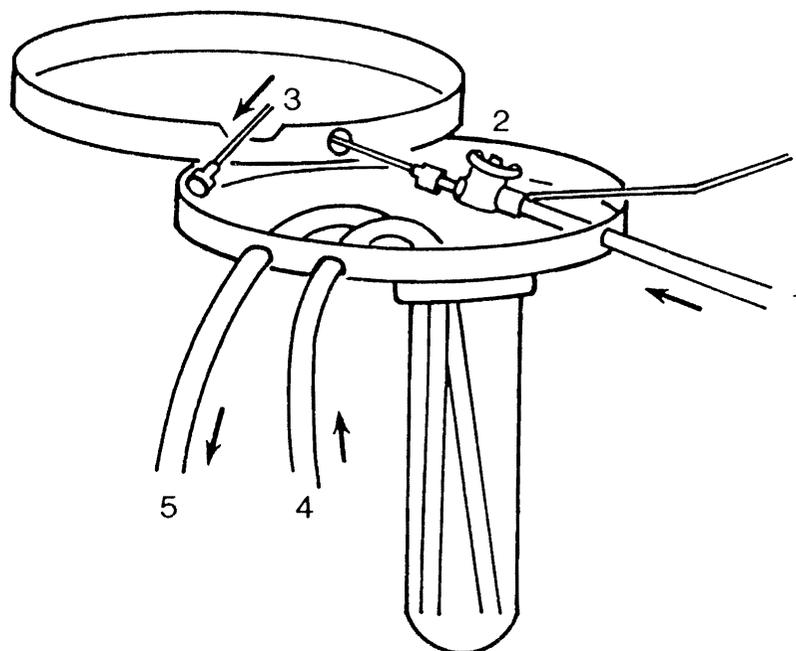
generator tubing is thin-walled silicon (T 5715-6, American Scientist, Warrendale, PA), which yields a partial pressure of oxygen of 500–600 mm Hg in each perfusion circuit when provided at 5 L/min of 95% O<sub>2</sub>/5% CO<sub>2</sub>.

#### *The Perfusate Flow Regulator*

A Master Flex pump controller (Model 7016-20) with silicon tubing (No. 6411-16 or 6411-14, Cole Parmer Instrument Co., Chicago, IL) is used in each of the independent perfusion systems. The flow rate of perfusate is kept at 2.5–3.5 mL/g liver min<sup>-1</sup>. The perfusate used is blood-free Krebs buffer with 2% bovine serum albumin. This allows measurements of oxygen consumption and release of various intracellular enzymes to be performed without interference from red blood cells. Higher rates of flow rapidly increase the pressure within the circuit and cause the liver to swell presumably secondary to vascular barotrauma. The percentage of prehepatic oxygen that is extracted is stable with minimal acidosis throughout at least 2½ h of perfusion.

#### *Perfusion Pressure Monitor*

The pressure within each perfusate circuit is continuously monitored through a three-way stopcock placed immediately before the portal vein cannula (Fig 1).<sup>5</sup> This is connected to a pressure manometer (No. 4607, Abbott Laboratories, North Chi-



**Figure 2.** Close-up diagram of liver platform and collection basin: 1, perfusate inflow; 2, three-way stopcock connected with manometer and portal vein cannula; 3, IVC cannula; 4, perfusate bypass; 5, perfusate outflows. The platform is made from petri dishes. Another dish placed upside down covers the platform.

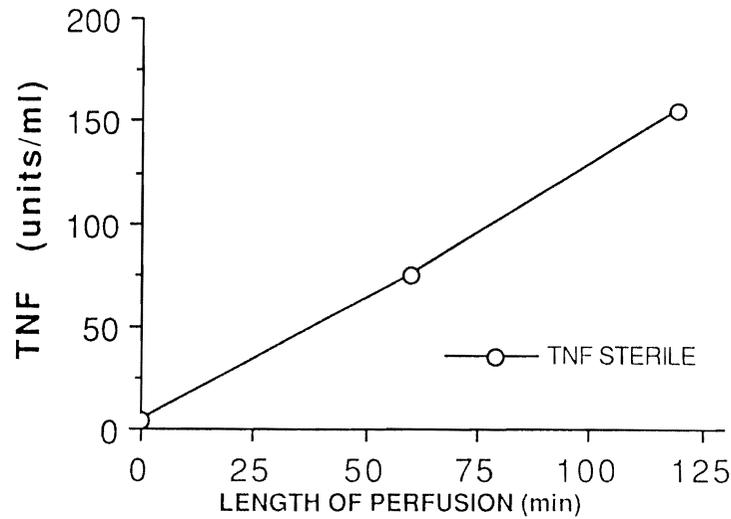
cago, IL), which is mounted on the side of the cabinet. Normal perfusion pressure for a control perfusion is 10–14 cm H<sub>2</sub>O. After a period of ischemia (cold or warm), it is important to slowly increase the rate of perfusate flow while keeping the pressure below 15 cm H<sub>2</sub>O. Too rapid resumption of the normal rate of flow will lead to greatly increased perfusion pressures, swelling of the liver, and release of hepatocellular enzymes.

#### ***Bubble Trap and Perfusion Filter***

We use a macropore filter (Blood set 64, Abbott) as both a bubble trap and perfusate filter. A filter is placed in-line within each inflow circuit and is disposed of after each perfusion. Removal of this disposable filter permits a more thorough irrigation of the tubing after each perfusion and simplifies sterilization.

#### ***Collection Basin for IVC Effluent***

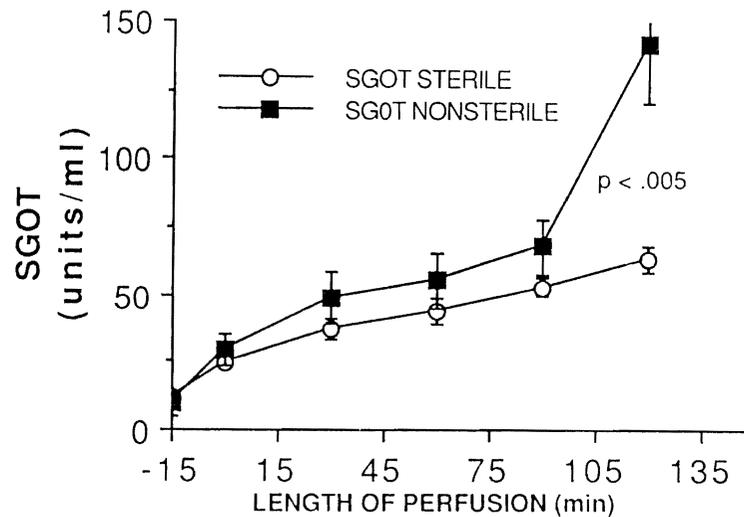
A 50-mL disposable sterile centrifuge tube (Corning Glass Works, Corning, NY) is attached to a petri dish (No. 5-757-13, Fisher Scientific Co.) in which an appropriately sized opening has been cut (Fig 2). Silicone Sealant (Bostik 9732, Middletown, MA) is used. This collection basin should also be covered with another inverted petri dish to assure a closed perfusion system and to avoid contamination during the perfusion. If the height of the centrifuge tube is adjusted properly, it can lie within the water bath, which helps to maintain the perfusate at the desired temperature.



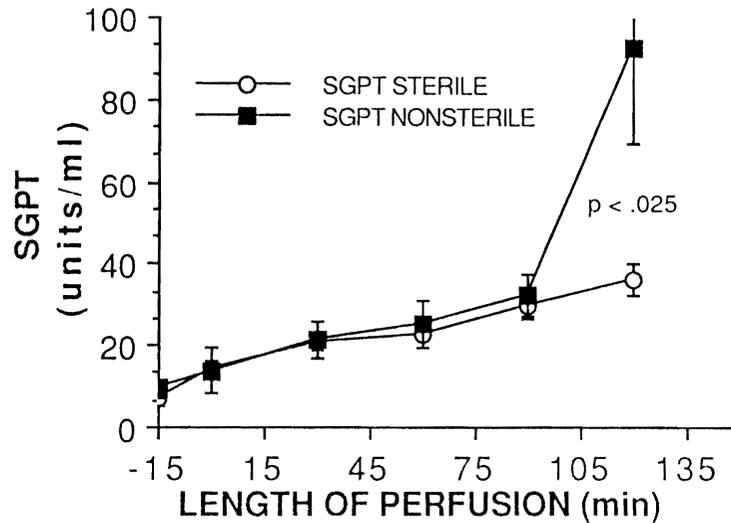
**Figure 3.** TNF production during sterile perfusion. TNF rises gradually during 120 min of perfusion due to recirculation and mild Kupffer cell activation.

#### *Sterilization of the Perfusion System*

During all surgical procedures and the assembly of the perfusion apparatus, a surgical mask and gloves are worn. A sterile pack that contains towels, 4 × 4 gauze pads, and cotton applicators is available for each liver harvest. Between perfusions, the basin for collection of IVC effluent and its cover are immersed in 1:750 Benz-All aqueous solution (XTTRIUM Lab, Chicago, IL) for at least 15 min and then rinsed with sterile water. The sterile blood set filter is opened immediately prior to the perfusion,

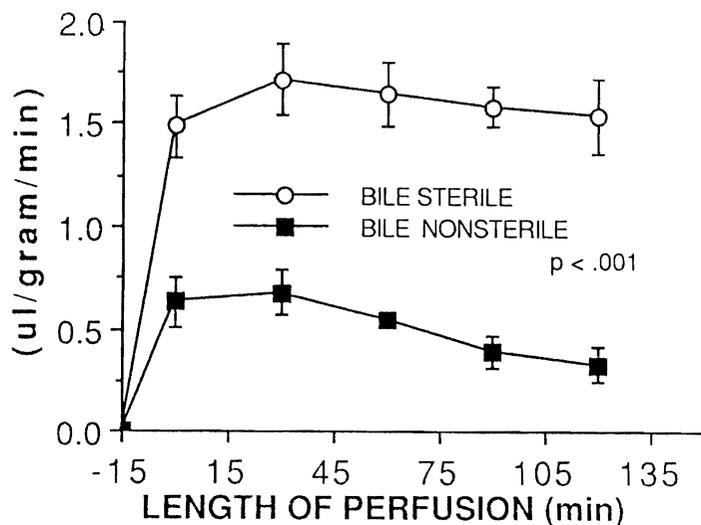


**Figure 4.** SGOT production during sterile and nonsterile perfusions. SGOT gradually rises in a linear fashion due to a recirculation in each system. After 90 min of perfusion, there is a large leakage of SGOT in the nonsterile perfusions. ( $N = 6$  sterile and 5 nonsterile perfusions.)



**Figure 5.** SGPT production during sterile and nonsterile perfusions. SGPT rises minimally during 120 min of sterile perfusion. During the nonsterile perfusions, there is a great rise in SGPT leakage after 90 min. ( $N = 6$  sterile perfusions.)

and the ends of the tubing and connectors (three-way stopcocks) are wiped with gauze saturated with 70% alcohol during assembly. The ports of the stopcocks should also be wiped with alcohol when a sample of perfusate is collected. The lumen of the apparatus tubing is disinfected by perfusion first with 10% Clorox (Oakland, CA) for 10 min. Then, 1 L of 0.9% sodium chloride is used to irrigate each perfusion circuit. When the tubing is empty, the collection basin is filled with sterilized Krebs buffer



**Figure 6.** Bile production during sterile and nonsterile perfusions. Bile production is much higher in the modified IPRL system and stays stable up to 180 min. ( $N = 6$  sterile and 5 nonsterile perfusions.)

solution containing 0.5 g of cefamandol/250 mL of perfusate (Eli Lilly, Indianapolis, IN).

Samples for culture are taken after 10 min by injection of 1 mL of perfusate into trypticase soy agar with 5% sheep blood (Becton Dickinson, Cockeysville, MD). We have found that, if we follow each of the above steps, an average of 15, and up to 20, consecutive perfusions with the same tubing will be sterile. The production of TNF, SGOT, and SGPT (Figs 3–5) and bile production (Fig 6) are reproducible. There is a gradual rise over small ranges in levels of TNF, SGOT, and SGPT due to recirculation. The leakage of enzymes is much less than before modification of the apparatus (Figs 3–6), especially after the first 90 min of reperfusion. Hepatocytes and nonparenchymal cells are intact histologically throughout 120 min of perfusion.

### Advantages of the Modified Perfusion System

1. The perfusion system is further simplified and made convenient to use and maintain because of a disposable inflow filter and a bubble trap that is inexpensive and commercially available.
2. The 95% O<sub>2</sub>/5% CO<sub>2</sub> mixture is more efficiently used, because two simultaneous perfusions in parallel can be completed in less time than it takes to do two separate sequential perfusions.
3. The length of tubing used for each perfusion is kept at a minimum, because the collection basin efficiently warms the perfusate. This simplifies the process of sterilization and lessens the cost of the apparatus.
4. The effluent collection basin and its cover are easily disinfected, which makes this a closed perfusion system. This avoids contamination during the perfusion.

In summary, we have improved the isolated rat liver perfusion system such that not only can two organs be perfused simultaneously, but the tubing and perfusates can be reliably sterilized for up to 20 consecutive perfusions. A gradual rise in transaminase levels occurs over a small range due to recirculation. Bile production is greatly increased over perfusions done before the present modifications and is stable throughout a perfusion.

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