A simple and inexpensive membrane "lung" for small organ perfusion

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Summary A disposable coil of thin-walled Silastic tubing, permeable to oxygen and carbon dioxide, functioned as a "lung" for perfusion of isolated rat liver. This "lung," which can be easily assembled from laboratory supplies, has a number of advantages over devices that utilize large air–liquid interfaces for gas exchange. Rates of hepatic lipoprotein triglyceride secretion, comparable to those obtained with more complex oxygenation systems, are achieved with this simple device.

Supplementary key words rat liver · gas exchange · oxygenation · disposable Silastic tubing · triglyceride production · lipoprotein secretion

A critical aspect of isolated organ perfusion is the maintenance of an adequate oxygen supply. In most of the gas-exchange systems that have been devised for this purpose, the perfusion medium is aerated as a thin film spread over a large surface area exposed to a stream of gas (1, 2). Because of the risk that plasma lipoproteins might be denatured at liquid–gas interfaces (3, 4), we considered that this method of oxygenation might be unsuitable for our perfusion studies, which were undertaken to investigate the structure of nascent plasma lipoproteins produced by rat liver. We decided, therefore, to use a Silastic membrane "lung" because such systems have been shown to accomplish efficient gas exchange (5). However, we found that the Silastic membrane oxygenators described in the literature or available commercially were too complex and costly for our requirements. Accordingly, we designed a simple and inexpensive membrane "lung" utilizing disposable Silastic tubing. A description of this device is given in this paper.

Materials and methods

The "lung" (Fig. 1) consists of a round specimen jar of about 500-ml capacity, approximately 110 mm high and 90 mm in diameter. Four ports are made in the lid by drilling holes and inserting plastic fittings. Two of these fittings (Fig. 1, A_1 and A_2) are standard polypropylene tubing connectors that fit tubing of 0.25 inch ID. The

TABLE 1. Gas exchange in perfusion medium during a single passage through the "lung"

Flow Rate of Medium	Silastic Tubing Length	Influent pO_2	Effluent pO_2	Influent pCO_2	Effluent pCO_2
ml/min	*ft*	*mm Hg*		*mm Hg*	
10.0	15	45 ± 2[a]	461 ± 9	45 ± 2	30 ± 1
12.1	12	15[b]	660	41	19
23.6	12	35	500	41	27
46.3	12	15	465	36	27
12.1	24	30	680	36	14
23.6	24	30	610	37	16
46.3	24	30	530	37	22

[a] The gas-exchange data in this row are the average values ± SEM for 18 experiments in which livers were perfused with balanced bicarbonate–saline medium containing erythrocytes at a hematocrit of 22%.

[b] The remaining data are from individual experiments in which bicarbonate–saline was first equilibrated with 95% N_2–5% CO_2 and then passed once through the "lung" tubing by means of a motor-driven syringe. The "lung" was flushed with 100% O_2 at 1.5 liters/min.

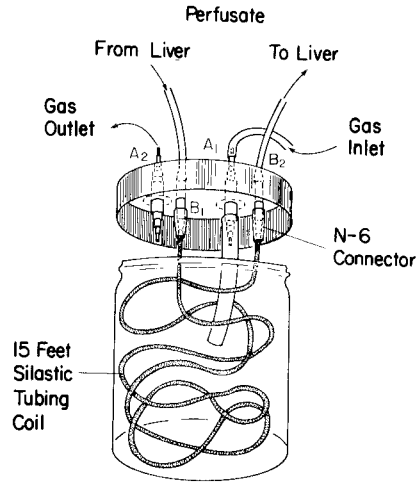

Fig. 1. Schematic drawing of the "lung." The gas mixture enters the jar through A_1 and is vented through A_2. The perfusion medium enters through B_1 and exits through B_2. The Silastic tubing (12–15 ft) is attached to connectors B_1 and B_2 by means of small polypropylene nipples and latex sleeving. In normal operation the lid is tightly sealed and a gas flow of 1–2 liters/min is maintained.

other pair of fittings (nylon needle adapters, Clay Adams, New York, cat. no. A-1001), shown in Fig. 1 as B_1 and B_2, accomodates latex rubber tubing (Will Ross Inc., Milwaukee, Wis., 0.125 inch ID, cat. no. 76-0066). The use of two different types of connectors avoids confusion when attaching tubing.

The gas mixture enters the jar through A_1, passes to the bottom of the container through a short piece of tubing, and is vented through A_2 at a flow rate of 1–2 liters/min. The perfusion medium enters through B_1, passes through Silastic tubing (12–15 ft), which is coiled loosely in the specimen jar, and exits through B_2. Coiling the tubing reportedly leads to significant improvement in gas transfer because of induced secondary velocities (6). It is unnecessary to support the coil on a frame. The medium is oxygenated during transit through this coil before passing to the liver. Hence, because gas exchange takes place through the walls of the coil, the dimensions of the Silastic tubing are critical. After preliminary studies we found that Silastic tubing of 1.47 mm ID and 0.25 mm wall thickness (Dow Corning Corp., Midland, Mich., cat. no. 602-235) was optimal. It is conveniently attached to connectors B_1 and B_2 within the jar by means of small polypropylene nipples (Technicon Instruments Corp., Tarrytown, N.Y., N-6, cat. no. 116-004-01) and latex sleeving (Fig. 1). Thicker-walled Silastic tubing did not provide adequate gas exchange, whereas thinner-walled tubing was too difficult to coil without kinking.

All tubing was routinely flushed with 500–1000 ml of saline to remove any trace chemicals that might contaminate the perfusate. Direct measurements of perfusate pO_2 and pCO_2 were made with appropriate electrodes of a blood-gas analyzer (Radiometer, Copenhagen, Denmark) and O_2 content was determined in a Lex-O_2-Con (Lexington Instruments Corp., Waltham, Mass.).

Results

For studies of plasma lipoprotein synthesis we used a balanced bicarbonate–saline medium (7) containing washed rat erthyrocytes at a hematocrit of 22%. These studies were performed on livers from male rats of the Long-Evans strain (350 g) fed ad lib. The average liver weight of these animals was 12 g, and a flow rate of 10 ml/min was sufficient for adequate oxygenation. The mean pO_2 of the medium leaving the liver was 45 ± 2 mm Hg (Table 1) and its O_2 content was 4.0 vol %. A single passage of this medium through the "lung" raised the pO_2 to 460 ± 10 mm Hg and the O_2 content to 10.2 vol % (averages of 18 experiments). From these data a mean hepatic O_2 consumption of 0.62 ml/min, i.e., 2.3 μmoles/g wet weight/min, can be calculated. This value is closely similar to that obtained in other perfusion studies in which film-type oxygenators were used (8, 9).

The mean pCO_2 of the perfusion medium was found to depend on both the rate of hepatic CO_2 production and the CO_2 tension of the mixture used for gassing. With the large livers used for studies of lipoprotein synthesis, there was no need to include CO_2 in the gas mixture during perfusion. The "lung" gassed with 100% O_2 lowered the pCO_2 of the perfusate 15 ± 2 mm Hg in a single passage, and this was balanced by metabolic CO_2 production so that a pCO_2 of 30 ± 1 mm Hg was maintained in the perfusate entering the liver (Table 1). Therefore, we initially equilibrated the perfusion medium with 95% O_2–5% CO_2 but changed the gas mixture to 100% O_2 as soon as the liver was placed in the circuit. If smaller

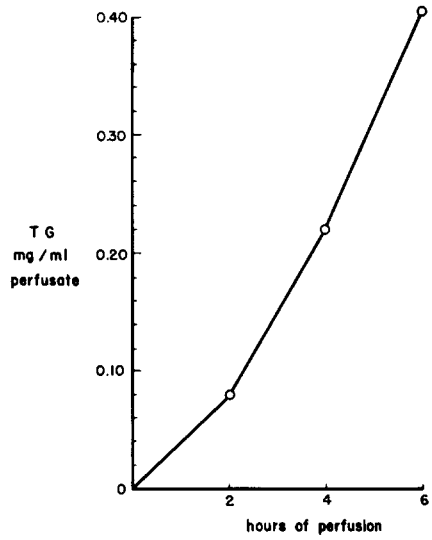

Fig. 2. Accumulation of triglycerides (TG) in perfusate. Livers of fed rats (350–375 g body wt) were perfused with about 60 ml of medium containing washed rat erythrocytes (22% hematocrit), 150 mg/100 ml glucose, and bicarbonate buffer (7). Each value is the mean of three experiments. The quantity and increasing rate of TG release into the perfusate is closely similar to the findings of others using a similar perfusion medium (containing bovine serum albumin) and a film-type oxygenator (10).

livers are perfused so that less metabolic CO_2 is generated, the "lung" should be flushed continuously with a gas mixture containing CO_2 in order to maintain a physiological pCO_2 in the perfusate. The optimum pCO_2 for the gas mixture is best determined experimentally.

We have used this "lung" routinely in maintaining perfused rat livers for 6 hr in order to obtain sufficient nascent high density lipoprotein for chemical characterization. In the presence of the erythrocyte-containing medium, yields of 1.5–3.0 mg of very low density lipoprotein (VLDL) protein and 0.3–0.6 mg of high density lipoprotein protein per liver are obtained. These hepatic perfusate lipoproteins have normal lipid and polypeptide compositions.[1] Triglyceride production, which reflects VLDL secretion, occurs at a linear or accelerating rate during the 6-hr perfusion (Fig. 2). Triglyceride secretion rates are comparable to those obtained by others using film-type oxygenators (10) and serum or albumin in the perfusates. Remarkably, in our experiments livers perfused for 6 hr show normal architecture with intact sinusoidal endothelium and apparently normal ultrastructure by both light and electron microscopy (Fig. 3). Swelling is not observed even though the perfusate contained no added albumin or other oncotic agent.

The "lung" is also used in a perfusion system for the preparation of intact isolated liver cells (11). In this technique the liver is perfused with erythrocyte-free medium. Data on the performance of the "lung" using cell-free medium are included in Table 1. A flow rate of 40–50 ml/min and a tubing length of 24 ft are used to ensure adequate oxygenation of the liver. Resistance to fluid flow through the "lung" tubing is reduced by using two parallel 12-ft coils rather than a single 24-ft length.

[1] Hamilton, R. L., M. C. Williams, R. J. Havel, and C. J. Fielding. Unpublished observations.

Discussion

The "lung" described in this paper has certain advantages over other oxygenation devices commonly used for liver perfusion studies. Both falling-film (1) and rotating-drum (2) oxygenators utilize an air–liquid interface in order to obtain adequate gas exchange. This arrangement may lead to evaporation of the medium and may also cause damage to cellular and protein elements of the blood (5, 12–15). The use of a membrane oxygenator avoids the problem of evaporation and should reduce the risk of mechanical trauma by eliminating the air–liquid interface. For example, the rate of hemolysis in our system is only 0.7% over 6 hr even though we use washed erythrocytes and no added serum proteins. The use of Silastic membrane systems for gas exchange is well established, and a number of efficient oxygenators have been developed (for review see Ref. 5). However, none of these systems was designed with the relatively simple needs of rat liver (or small organ) perfusion in mind. Likewise, the membrane oxygenators available commercially are not well suited for small organ perfusion. They are extremely expensive, require a large priming volume, and their complexity makes thorough cleaning difficult and time-consuming.

The device we describe is both inexpensive and simple to construct from laboratory supplies. Its fabrication requires neither the skills of an expert glassblower nor the facilities of a machine shop. Only a small quantity of perfusate is needed to prime the system. In fact, since the capacity of the Silastic tubing is only 8–10 ml (0.7 ml/ft), the perfusate volume can be reduced to a value of the same order as that of the total blood volume for a 350-g rat (ca. 25 ml). Moreover, the small size of the device permits a substantial reduction in the size of the perfusion cabinet. We perfuse two livers, side by side, in a cabinet only 45 × 45 × 40 cm. We have not been concerned with collecting metabolic CO_2 in studies involving the production of $^{14}CO_2$ from ^{14}C-labeled substrate. However, it is apparent that the device can be easily adapted for such studies by merely passing the vented gas through a CO_2 trap. The "lung" tubing is disposable and is sufficiently inexpensive that it can be replaced for each individual experiment, thereby avoiding the problems of cleaning. Moreover, sterilization is readily accomplished.

The Silastic tubing "lung" appears to be the system of choice for small organ perfusion. In our hands, no other system has permitted the perfusion of rat livers for 6 hr with an albumin-free medium while avoiding hepatic

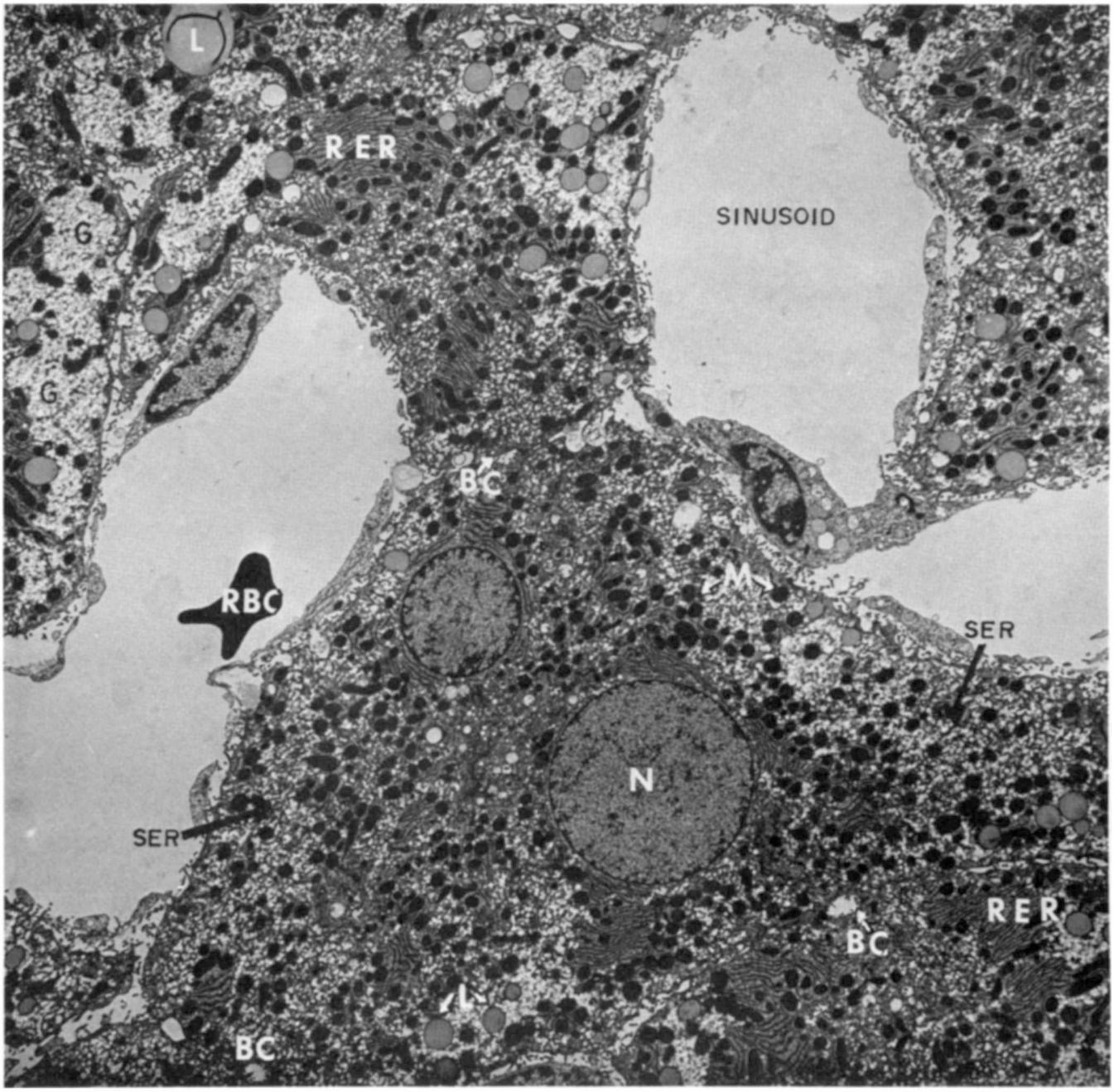

Fig. 3. This low-magnification (× 4000) electron micrograph demonstrates the appearance of rat liver perfused for 6 hr in the perfusion apparatus that utilizes the Silastic tubing "lung." The normal architecture of the hepatic parenchymal cells and adjacent sinusoids appears relatively undisturbed by the perfusion. The endothelial and Kupffer cells form a discontinuous lining that overlies the microvillous surface of the hepatic cells. The bile canaliculi (*BC*) and their associated junctional complexes remain intact. Parenchymal cells contain intact nuclei (*N*), parallel arrays of rough-surfaced endoplasmic reticulum (*RER*), meshworks of smooth-surfaced endoplasmic reticulum (*SER*), frequent lipid droplets (*L*), and mitochondria (*M*). The pale-staining areas of cytoplasm contained glycogen (*G*), which was extracted by the acidic uranyl acetate block stain. In the largest sinusoid is a red blood cell (*RBC*). The tissue was fixed by perfusion through the portal vein with a mixture of glutaraldehyde and paraformaldehyde in a phosphate buffer. Tissue blocks were then postfixed in 2% osmium tetroxide, stained in buffered uranyl acetate, dehydrated in acetone, and embedded in Epon. Thin sections were stained for electron microscopy with an alkaline solution of lead citrate.

edema and microscopic evidence of cell damage. The presence of serum proteins in concentrations used by others in similar studies (1, 2, 4, 8–10) does not diminish the efficiency of gas exchange by the "lung" during 6 hr of perfusion. Results obtained with the described perfusion medium supplemented with 4 g/dl crystalline bovine serum albumin or another medium containing 3 parts rat serum mixed with washed rat erythrocytes are closely similar to

those shown in Table 1. It is hoped this description will encourage other investigators to try the device for small organ perfusion studies.

Since our manuscript was submitted, we have seen a description of a similar device in a preliminary report (16). Although the details of construction and the data provided were comparatively sparse, it is evident that these investigators have successfully applied this simple technique for the perfusion of the hind limb of the rat.

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REFERENCES

1. Miller, L. L., C. G. Bly, M. L. Watson, and W. F. Bale. 1951. The dominant role of the liver in plasma protein synthesis. A direct study of the isolated perfused rat liver with the aid of lysine- ϵ-C^{14}. *J. Exp. Med.* **94:** 431–453.
2. Mortimore, G. E. 1961. Effect of insulin on potassium transfer in isolated rat liver. *Amer. J. Physiol.* **200:** 1315–1319.
3. Zapol, W. M., R. I. Levy, T. Kolobow, R. Spragg, and R. L. Bowman. 1969. In vitro denaturation of plasma α-lipoproteins by bubble oxygenation in the dog. *Curr. Top. Surg. Res.* **1:** 449–467.
4. Windmueller, H. G., and A. E. Spaeth. 1972. Fat transport and lymph and plasma lipoprotein biosynthesis by isolated intestine. *J. Lipid Res.* **13:** 92–105.
5. Melrose, D. G., N. Burns, M. P. Singh, R. L. Elliot, R. Read, F. E. Williams, J. Becket, M. P. Lamb, and J. S. Adams. 1972. Oscillating silicone membrane tubes: a new principle of extracorporeal respiration. *Biomed. Eng.* **7:** 60–66.
6. Weissman, M. H., and L. F. Mockrus. 1968. Gas transfer to blood flowing in coiled circular tubes. *J. Eng. Mech. Div. Proc. Amer. Soc. Civil Eng.* **94:** 857–872.
7. Krebs, H. A., and K. Henseleit. 1932. Untersuchungen über die Harnstoffbildung im Tierkörper. *Hoppe-Seyler's Z. Physiol. Chem.* **210:** 33–66.
8. Hems, R., B. D. Ross, M. N. Berry, and H. A. Krebs. 1966. Gluconeogenesis in the perfused rat liver. *Biochem. J.* **101:** 284–292.
9. Exton, J. H., J. G. Corbin, and S. C. Harper. 1972. Control of gluconeogenesis in liver. V. Effects of fasting, diabetes, and glucagon on lactate and endogenous metabolism in the perfused rat liver. *J. Biol. Chem.* **247:** 4996–5003.
10. Windmueller, H. G., and A. E. Spaeth. 1967. De novo synthesis of fatty acid in perfused rat liver as a determinant of plasma lipoprotein production. *Arch. Biochem. Biophys.* **122:** 362–369.
11. Berry, M. N., and D. S. Friend. 1969. High-yield preparation of isolated rat liver parenchymal cells. A biochemical and fine structural study. *J. Cell Biol.* **43:** 506–520.
12. Landé, A. J., S. J. Dos, R. G. Carlson, R. A. Perschau, R. P. Lange, L. J. Sonstegard, and C. W. Lillehei. 1967. A new membrane oxygenator–dialyzer. *Surg. Clin. N. Amer.* **47:** 1461–1470.
13. Kolobow, T., W. Zapol, and J. Marcus. 1968. Development of a disposable membrane lung for organ perfusion. *In* Organ Perfusion and Preservation. J. C. Norman, editor. Appleton-Century-Crofts, New York. 155–175.
14. Vervloet, A. F. C., M. J. Edwards, M. L. Edwards, and A. Starr. 1970. Minimal apparent blood damage in Landé-Edwards membrane oxygenator at physiologic gas tensions. *J. Thorac. Cardiovasc. Surg.* **60:** 774–780.
15. Ekert, H., D. Montgomery, and E. Aberdeen. 1971. Fibrinolysis during extracorporeal circulation: comparison of the effects of disc and membrane oxygenators. *Circ. Res.* **28:** 512–517.
16. Finseth, F., H. Hattori, and J. F. Burke. 1972. Perfusion of an isolated capillary system for the analysis of capillary transport phenomena. *J. Trauma.* **12:** 75–80.