Naturally, therefore, the metabolic uptake of DCMU is an outcome of a mutational event. The ability of the $DCMU^r$ mutant to metabolize up to 5.0×10^{-5} M DCMU might be responsible for relieving the organism from DCMU-induced cellular lysis, because the mutant obviously appears to have acquired resistance to both forms of DCMU-induced inhibition, i.e., photosynthetic inhibition and cellular lysis. It has probably lost the ability to utilize light energy, leading to the disappearence of the site of inhibition by 1.0×10^{-5} M DCMU. It is, therefore, unlikely that the same mutation can have conferred the ability to synthesize a novel DCMU-metabolizing enzyme system. A possible explanation is that parent *Nostoc muscorum* and the $DCMU^r$ mutant derived from it can both form enzymes capable of metabolizing DCMU, but DCMU can be utilized only when its intracellular level rises above the level which inhibits the photosynthetic assimilation of CO_2. This suggestion is supported by the finding that while DCMU was toxic to the parent organism at a concentration of 1.0×10^{-5} M, it was metabolized by the $DCMU^r$ and MSO^r mutant strains only at concentrations above 1.0×10^{-5} M. Nevertheless, as a readily-metabolizable nitrogen source (as discussed above), DCMU suppressed the formation of heterocysts and supported the increased phycocyanin-yield in both $DCMU^r$ and MSO^r mutants at concentrations strictly above 1.0×10^{-5} M.

MSO is a well known inhibitor of GS activity in blue-green algae[15]. It has been found that a mutation conferring resistance to 3.70×10^{-4} M MSO is associated with an altered GS having higher resistance to MSO inhibition in comparison to the enzyme from the MSO-sensitive parent *N. muscorum*[8]. The present findings that the MSO^r mutant showed a certain degree of cross-resistance for DCMU and vice-versa, suggest that possibly a common site is involved in interactions with DCMU and MSO. The reason why 2.0×10^{-5} M DCMU did not inhibit the growth of the MSO^r strain (as it did in the MSO-sensitive parental strain), may be that a mutation leading to an altered GS (having a higher resistance to MSO) might have been associated with an increased affinity of GS for DCMU. Such an increased affinity leading to the utilization of low concentrations of DCMU might have resulted in the MSO^r mutant having a lower intracellular concentration of DCMU than the parent organism (when the external concentration of DCMU was 1.0×10^{-5} M).

In *Nostoc muscorum* there have been indications of the possible involvement of GS in the metabolism of substrate analogues like methylamine, as a carbon and nitrogen source[10]. It has also been worked out that GS from bacterial[16-19] and eukaryotic[20,21] cells can metabolize methylamine (like ammonia) to some extent. This is further confirmed through radioactive tracer studies[22,23]. It is, therefore, strongly believed that the $DCMU^r$ mutant of *N. muscorum*, as a result of a defect in the photosynthetic mechanism, is capable of utilizing DCMU (containing a methylurea group) as a carbon and nitrogen source (like methylamine) by virtue of an existing enzyme system which may possibly be GS.

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A new simple temperature-controlled membrane oxygenator for the perfusion of isolated rat livers

M. Ogura, Y. Yamamoto and Y. Ogura[1]

Department of Biochemistry, Tottori University School of Medicine, Yonago, Tottori 683 (Japan), 2 March 1984

Summary. A new temperature-controlled membrane oxygenator for perfusing isolated rat livers was assembled using a combination of heat-exchangeable rubber tubing and silicon rubber tubing. The apparatus supplied enough oxygen to satisfy the requirements of hemoglobin-free perfused livers.
Key words. Membrane oxygenator; perfusion of rat livers.

In an organ-perfusion system, the oxygenator is an essential component. There are two principal types of oxygenators. In one type, the perfusate is oxygenated at large fluid-gas interfaces[2-4], and in the second, oxygen is transferred by diffusion through a membrane and dispersion within the flowing perfusion medium[5-8]. The latter type of oxygenator has some advantages over the former[6,8].

Most perfusion systems employ a thermostatically regulated cabinet with an oxygenator in it. Some research workers[5,9], however, have used a temperature-controlled membrane oxy-

genator without any such cabinet. The authors have designed a new model of this type of oxygenator. It is different in structure from the membrane oxygenators described so far, but comparable with them in function.

The inside of a piece of heat-exchangeable synthetic rubber tubing (length 18 m, width of the contact surface 8 mm, Calorex) was rinsed with a dilute solution of a neutral detergent and then with water, and dried thoroughly by aeration. A piece of silicon rubber tubing about 18.5 m long (2 mm inner diameter, 0.2 mm wall thickness; Detakta) was passed through the Calorex tubing.

Two frames were made of acrylic materials. A pair of pipes (20 mm outer diameter, 1.5 mm wall thickness, 230 mm length) and a pair of plates (250 × 30 × 3 mm) were arranged in parallel crosses, in which the same parts faced each other on opposite sides. The Calorex tubing with the silicon tubing within its lumen was wound closely in a single layer around the parallel pipes of the frames. The frames with the wound tubing were bolted on to an acrylic plate (290 × 275 × 3 mm) like a two-layered rack. The space between the plate and the frames was fixed by the lengths of small pieces of acrylic tube which were put on the bolts.

The ends of the Calorex tubing were led through two holes on the plate to the reverse side of the frames and each end was connected with either a gas inlet/fluid outlet (GI-FO) or a gas outlet/fluid inlet (GO-FI) device. These devices were of the same structure, consisting of a glass tube (20 mm outer diameter and 70 mm length) with both ends open, fitted in the middle with a side arm (6 mm outer diameter) and at the opposite side with a leg for fixing on to the plate. A pair of silicon rubber stoppers (No. 4) was fitted into both ends. Each stopper had a port which was made by drilling a hole and inserting a teflon tube A (6 mm outer diameter and 1 mm wall thickness) or a teflon tube B (4 mm outer diameter and 0.5 mm wall thickness). Both ends of the Calorex tubing were connected with the tube A, and both ends of the silicon tubing coming out from the Calorex tubing were connected with the tube B within the device, after some portions had been cut off to adjust the length. The two devices were fixed by their legs in the central part of the plate in parallel with each other.

The assembly is placed in a temperature-controlled water bath so that the Calorex tubing wound on the frames is completely submerged in the water. The plate with the devices on it, fitting the edge of the bath, serves as a cover. A gas mixture of O_2/CO_2 (95:5) enters the apparatus through the side arm of the GI-FO device, passes to the GO-FI device through the space between the Calorex tubing and the silicon rubber tubing, and leaves the device through its side arm at a flow rate of 0.5 l/min. The perfusion medium pumped from a reservoir enters through the teflon tube B of the GO-FI device, passes through the silicon tubing placed in the Calorex tubing, and exits through the teflon tube B of the GI-FO device. The table shows the results of oxygenation experiments with the apparatus. Under the conditions indicated, the perfusion medium was oxygenated with gases at 1 atm up to the theoretically maximal level after passing once through the apparatus, and was maintained at a constant temperature when the flow rate was fixed.

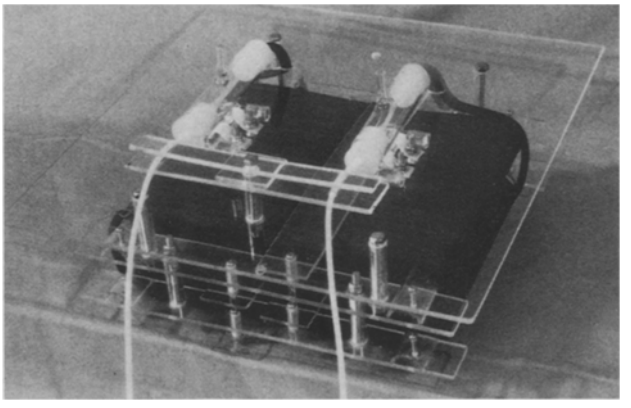

Figure 1. Whole apparatus.

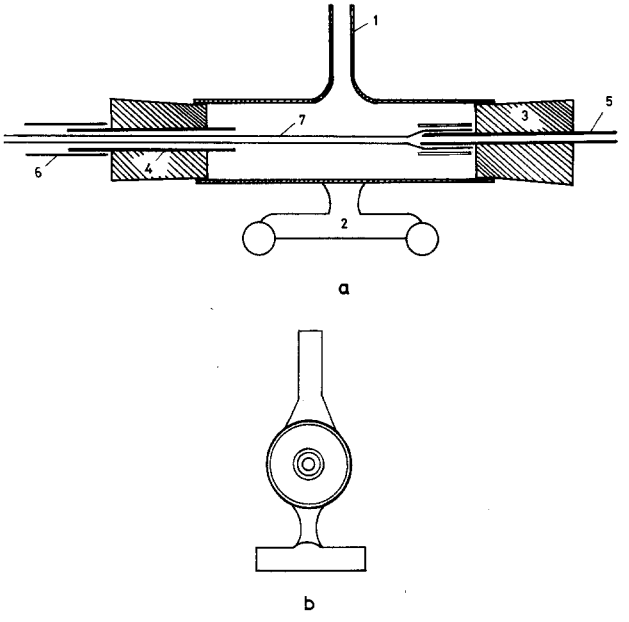

Figure 2. Device for gas inlet/fluid outlet (or gas outlet/fluid inlet). *a* side view; *b* front view. 1, side arm; 2, leg for fixing; 3, silicon rubber stopper; 4, teflon tube A; 5, teflon tube B; 6, Calorex tubing; 7, silicon rubber tubing.

Temperature and pO_2 of the perfusion medium after passage through the apparatus

Room temperature (°C)	Oxygenating gas (flow rate, 0.5 l/min at 1 atm)	Perfusion medium Water or KHB*	Flow rate (ml/min)	Temperature** (°C)	pO_2 (mm Hg)	% of theoretical maximum
22	O_2:CO_2 (95:5)	Water	35	22***	711.6	101.2
22	O_2	Water	35	22***	724.0	97.8
25	O_2:CO_2 (95:5)	Water	20	35.4	671.4	98.6
25	O_2:CO_2 (95:5)	Water	30	36	687.3	101.1
25	O_2:CO_2 (95:5)	Water	35.5	36	688.2	101.3
25	O_2:CO_2 (95:5)	Water	39.5	36.5	696.4	102.6
25	O_2:CO_2 (95:5)	Water	48	36.9	695.5	102.7
23	O_2:CO_2 (95:5)	KHB	36	36	701.8	103.3

* Krebs-Henseleit bicarbonate buffer (pH 7.4); ** measured after passing the oxygen electrode and adjusted to 36 °C at a flow rate of 35 ml/min; *** not controlled.

The maximal oxygen transfer with Krebs-Henseleit bicarbonate buffer (pH 7.4) at 36 °C and at a flow rate of 36 ml/min for example was 2.05 mmoles/h.

Male Wistar strain rats, weighing 250–300 g, fed on a commercial rat diet (Clea CE-2, Nihon Clea Co.) were anesthetized by i.p. injection of sodium pentobarbital (5 mg/100 g b.wt; Nembutal, Abbott Laboratories) and operated on for perfusion of their livers in the same way as described previously[10], except that the portal vein was cannulated before the inferior vena cava was ligated and divided. Livers were perfused with Krebs-Henseleit bicarbonate buffer (pH 7.4) supplemented with 5.5 mM glucose at a flow rate of about 40 ml/min in non-recirculating system, which was essentially based on the method of Scholz et al.[5] except that the oxygenator described above, regulated at 36 °C, was used. The hepatic oxygen consumption was measured according to a method previously reported[11].

Oxygen consumption and bile secretion in perfusions under the conditions employed were 159.6 ± 7.7 μmoles/h and 46.6 ± 5.6 mg/h per g of liver weight (mean ± SE, n = 9), respectively, during the first hour. These values were comparable to those obtained in other perfusion experiments with rat livers in which membrane as well as other types of oxygenators were used[6, 10, 12–14]. The initial level of the oxygen consumption was maintained even after 3 h of perfusion.

In summary, the apparatus described in this paper supplies the O_2-requirements of perfused rat livers well. It is inexpensive, and can be easily assembled without any specialized machine shop facilities.

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