Flux of metabolites and changes in glycogen concentrations during incubation of the EDL muscle

	First 30 min	Second 30 min	Thrid 30 min	
	Rest	Rest	Rest	
Glucose uptake	19.2 ± 1.69	21.2 ± 3.42	22.4 ± 2.08	(8)
Lactate output	5.3 ± 0.81	6.3 ± 1.08	7.0 ± 0.98	(8)
Acetoacetate uptake	1.4 ± 0.72	1.4 ± 1.76	1.5 ± 0.66	(8)
β-hydroxybutyrate output	1.0 ± 0.45	1.0 ± 0.33	0.9 ± 0.14	(8)
Glycogen	2.6 ± 0.24	1.6 ± 0.21	—	
	Rest	Activity	Post-activity	
Glucose uptake	19.7 ± 3.01	12.8 ± 2.68[a]	19.2 ± 3.13[b]	(9)
Lactate output	5.1 ± 0.61	7.6 ± 1.19[a]	5.9 ± 1.05	(9)
Acetoacetate uptake	0.8 ± 0.24	1.2 ± 0.30	1.2 ± 0.23	(9)
β-hydroxybutyrate output	0.7 ± 0.20	0.8 ± 0.28	1.0 ± 0.20	(9)
Glycogen	2.8 ± 0.14	2.1 ± 0.20[a]	—	(6)

Uptakes or production of metabolites are expressed as μmol/30 min/g wet wt. and glycogen concentrations at the end of the period as mg/g wet wt. Values given are means ± SEM. ( ) = number of animals. [a] Significant difference between first and second periods; [b] significant difference between second and third periods ($p < 0.05$, t-tests).

In order to make the muscle perform work, supramaximal isotonic twitches were produced by impulses of 60 V, 1 msec duration at a frequency of 0.25 pulse/sec. With this type of stimulation the muscle fatigued only about 40% during a 30 min period.

The following metabolites in the incubation medium were measured: glucose[5], lactate[6], acetoacetate and β-hydroxybutyrate[7]. Muscle glycogen was also measured[8].

Results. The isolated EDL muscle preparation was judged to be suitable for in vitro experimentation on the basis of the following criteria: 1. no changes in wet or dry weights were apparent after the muscle was incubated under slight tension for 105 min. 2. The macroscopic appearance did not change nor was there evidence of oedema formation. 3. After a 15 min incubation period in 0.02% Evans blue solution a uniform distribution of the dye was seen in a cross section using light microscopy. 4. After 105 min of incubation the K^+ concentration in the buffer increased by less than 5% and the pH remained between 7.3 and 7.4 after a 30 min rest or contraction period. 5. On incubation with insulin (1 munit/ml) the glucose uptake more than doubled.

In order to investigate muscle metabolism during consecutive 30 min periods of rest, activity and post-activity, it is important that biochemical changes in the muscle are consistent throughout three 30 min periods with the muscle at rest. The Table shows that there was no alteration in the flux of metabolites or muscle glycogen concentrations during consecutive rest periods. However, if the muscle was stimulated during the second period, changes in both the metabolite flux and glycogen levels were readily discernible.

Discussion. It is felt that the evidence presented demonstrates that the isolated EDL muscle is suitable for investigations of muscle metabolism during consecutive 30 min periods of rest, activity and post-activity. Unlike other more complex muscle preparations such as the perfused hind-quarter[9], the preparation was simple and incubated in apparatus that was easily constructed. Furthermore, and most important, this preparation enables skeletal muscle metabolism to be studied in its essential function of contraction, exercise and work, when the contribution to the body's energy utilization may be as great as 90% and cause profound effects on total body metabolic pathways.

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A Simple Membrane Oxygenator for the Isolated Rat Liver Perfusion

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Summary. Silastic capillary tubing was used in isolated rat liver perfusions as a simple membrane oxygenator supplying sufficient oxygen to cover the requirements of the liver.

A need of a sterilizable, re-usable, small, membrane oxygenator for rat liver perfusion has been expressed[1], because of the deleterious changes in blood produced by the simple film oxygenators. BODELL et al.[2] have used membrane oxygenators consisting of silastic capillary tubing in extracorporeal circulation experiments with sheep and FOLKMAN et al.[3] in isolated perfusion of rat thymus. In our rat liver perfusions, we have utilized the excellent O_2 and CO_2 transmission properties of silastic capillary tubing, without constructing any separate oxygenation unit.

Two 4-m lengths of Silastic Medical-Grade Tubing (i.d. 0.30 mm; o.d. 0.64 mm; Dow Corning Center for Aid to Medical Research, Midland, Michigan) were folded into a coil and inserted into a 50-cm length silastic tube with internal diameter of 6 mm. One end of the outside tube was directly connected to the inlet side of the perfusion pump and the other end to the perfusate reservoir so that the ends of the capillary tubes could be drawn to the outside through the perfusate reservoir. The oxygenating gas (95% O_2, 5% CO_2) was passed first into a glass bottle with two needles (No. 20) run through a rub-

Oxygen transport and pO_2, pCO_2, pH of the perfusion medium and the hepatic perfusate flow during rat liver perfusion

Time (min) from the beginning of the perfusion	0	30	60	90	120
Hepatic perfusate flow (ml/g liver/min)		2.1 ± 0.4	2.1 ± 0.4	2.1 ± 0.4	2.0 ± 0.4
pO_2 (mmHg)	226 ± 45	123 ± 46	125 ± 48	132 ± 40	145 ± 40
pCO_2 (mmHg)	18 ± 6	22 ± 6	23 ± 7	23 ± 7	22 ± 7
pH	7.44 ± 0.1	7.29 ± 0.07	7.23 ± 0.09	7.19 ± 0.08	7.16 ± 0.10
Oxygen transport					
ml/g liver/h		6.3	6.3	6.3	6.0
μmol/g liver/min		4.7	4.7	4.7	4.5

Values represent means ± SD of 40 perfusions.

ber stopper. The inlet ends of the capillary tubes were connected to the needles, the outlet ends were left free. A small overpressure for the gas flow through the capillary tubes was necessary and the rubber stopper worked as an escape valve, flying off if the gas flow was hindered. The total gas flow through the two capillary tubes was 50–60 ml/min.

In other respects the perfusion system was a modification of the general principles and apparatus presented by SEGLEN and JERVELL[4]. A total perfusate volume of 50 ml was employed, consisting of 12 ml heparinized fresh rat blood, 38 ml Krebs-Ringer bicarbonate buffer solution, 50 mg D-glucose and 1.25 g bovine serum albumin (fraction V, Armour Pharmaceutical Co., Eastborne). Hemoglobin level of the perfusion medium was about 35 g/l. The perfusion pressure was 16–17 cm H_2O and the temperature 37 °C. The pump flow rate was about twice the actual hepatic perfusion flow. pO_2, pCO_2 and pH were measured by a micromethod (Combi-Analyser, L. Eschweiler, Kiel, with electrodes Eak1 for O_2, Eak2 for CO_2, and Eak3 for pH).

In the Table are presented pO_2, pCO_2, pH and oxygen transport values of the perfusion medium at the inlet side of the liver, and the hepatic perfusate flow rates of 40 perfusions, oxygenated by means of the Silastic capillary tubing. One 4-m length of the tubing was not sufficient for the oxygenation. With two 4-m lengths of the tubing the medium obtained enough oxygen to supply the oxygen consumption of the liver. Taking into account the hepatic perfusate flow rate, hemoglobin level of the medium and assuming oxygen solubility to be 0.3 ml/100 ml, hemoglobin to bind 1.34 ml O_2/g, an oxygen transport value of about 4.7 μmol/g liver/min (6.3 ml/g liver/h) by the perfusion medium, could be calculated. This exceeds the reported oxygen consumption values presented by many authors[5]. BRAUER et al.[6] have found that the oxygen consumption of the rat liver under physiological conditions was 7.8 ml/g/h and in an erythrocyte free perfusate close to 2.0 ml/g/h. Increasing the number of tubings enlarges the gas exchange surface and raises the oxygen tension in the perfusion medium, thus increasing the danger of bubble formation and changes in hepatic tissue[7]. Higher hemoglobin level in the perfusion medium increases oxygen transport, but makes the perfusion medium more expensive. CO_2 retention did not occur. The pH decreased in the course of perfusions, as it does when no special measures are taken to control pH[8].

We feel that this method is very useful in all kinds of isolated organ perfusions where a simple membrane oxygenator is needed. The system is easy to assemble, use and sterilize. Our experience is that Silastic capillary tubing is durable and re-usable for tens of perfusions. Because it does not easily kink, it may be inserted into tubes connecting different units in organ perfusions, and the need for a separate oxygenator is eliminated.

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A New Synthesis of Benzoyl Phosphate: A Substrate for Acyl Phosphatase Assay[1]

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Summary. A new method for the synthesis of benzoyl phosphate was reported. The advantages are: 1. more rapid procedure; 2. lower cost; 3. higher yield.

Benzoyl phosphate has been shown to be a very useful substrate for the determination of acyl phosphatase activity. In fact it was shown that UV-absorption spectrum of benzoyl phosphate in the 220–300 nm region differs markedly from that of its hydrolysis product, i.e. benzoate and inorganic phosphate. This difference has been used by our and other laboratories[2–4] to make a continuous optical test for acyl phosphatase. Benzoyl

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