phic subpopulations if appropriate assay procedures are employed[3,4]. The single most important contribution of these findings is the observation that chromatography must be performed with various concentrations of the steroid vs the receptor to avoid negative or even erroneous conclusions such as have already appeared in other reports[6]. It is to be hoped that these considerations may lead to better understanding of the mechanism regulating steroid-target organ specificity. The most frustrating aspect, however, remains the technical limitations that have hitherto prevented unequivocal demonstration of a direct relationship between receptor activity and physiological action of the hormone in question.

[6] M. Beato and P. Feigelson, J. biol. Chem. *247*, 7890 (1972).

PRO EXPERIMENTIS

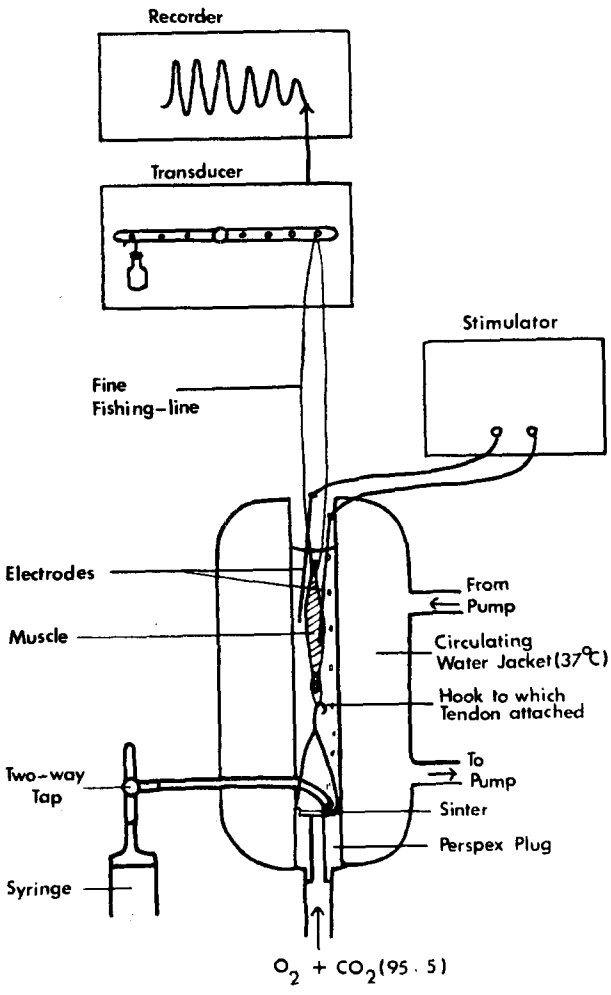
A Preparation for the Study of Muscle Metabolism During Rest and Activity

G. J. Partington[1] and J. K. Wales

University of Leeds, Department of Medicine, Leeds General Infirmary, Leeds LS1 3EX (England), 23 October 1975.

Summary. A method for the investigation of metabolism in the isolated rat extensor digitorum longus muscle has been described. This muscle was shown to be suitable for investigations of skeletal muscle metabolism during rest and activity.

In mammals skeletal muscle makes up about 40% of the body weight. Yet investigations into the biochemical properties of the muscle at rest and during exercise are comparatively rare. It has often been considered that whole muscles incubated in physiological solutions are not sufficiently thin to rely on diffusion of substrates and oxygen from the medicine. It is also difficult to catheterize the vessels of small laboratory animals if perfusion is considered as an alternative. The rat diaphragm, which is widely used for insulin assay[2], is clearly not representative of the bulk of skeletal muscle[3].



Apparatus to study isolated skeletal muscle metabolism.

The present study was undertaken to explore the suitability of the isolated rat extensor digitorum longus (EDL) muscle for studies of muscle metabolism during rest and activity. This muscle has many features which make it particularly suitable for in vitro experimentation: it is thin and cylindrical therefore allowing easy diffusion, the fibres are longitudinally arranged and there are long well-defined tendons at each end which can be ligatured without interfering with any of the muscle tissue, and as each animal yields 2 muscles a paired control technique can be used.

Materials and methods. Male Wistar rats weighing 240–260 g were killed by cervical fracture, and with minimal handling the EDL muscle was quickly removed and weighed. A small cotton loop was fixed to the distal tendon and a 10 cm length of fishing line (breaking strength 0.5 kg) fixed to the proximal tendon. Use of fishing line avoided the shrinkage found when cotton was placed in the perfusate. The muscle was transferred to the incubation chamber (Figure) containing 4 ml Krebs bicarbonate buffer[4]. A one-gram load was attached on the recording arm as a counterbalance and the resting muscle was adjusted so that it was slightly stretched by the load. In order to study muscle metabolism during rest, activity and post-activity periods the following incubation procedure was employed: 15 min preincubation, 30 min rest, 30 min stimulation and 30 min post-stimulation. After each period the medium was withdrawn and fresh medium added. In experiments involving measurement of intramuscular metabolites, 2 muscles were incubated in separate chambers and the metabolite concentrations could be determined in one muscle at the beginning of the period, and in the other at the end of the period.

[1] Recipient of a Medical Research Council Scholarship for training in research methods.

[2] A. F. Willebrands, J. Groen, E. Kamminga and J. R. Blickman, Science *112*, 277 (1950).

[3] R. D. Peterson, C. H. Beatty and R. M. Bocek, Am. J. Physiol. *200*, 182 (1961).

[4] W. W. Umbreit, R. H. Burris and J. F. Stauffer, in *Manometric Techniques* (Burgess Publishing Co., Minneapolis, Minn. 1957), p. 149.

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.

[5] G. MORLEY, A. DAWSON and V. MARKS, Proc. Ass. clin. Biochem. *5*, 42 (1968).
[6] G. F. OLSEN, Clin. Chem. *8*, 1 (1962).
[7] D. H. WILLIAMSON, J. MELLANBY and H. A. KREBS, Biochem. J. *82*, 90 (1962).
[8] F. HUIJING, Clin. chim. Acta *30*, 567 (1970).
[9] N. B. RUDERMAN, C. R. S. HOUGHTON and R. HEMS, Biochem. J. *124*, 639 (1971).

A Simple Membrane Oxygenator for the Isolated Rat Liver Perfusion

K. NEVASAARI

Department of Pharmacology, University of Oulu, SF-90 220 Oulu 22 (Finland), 22 September 1975.

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-