

the filter cool and place it in a 37°C oven until complete dehydration. The thin film (0.3–0.5 mm) of colored and dried Agar is carved to obtain a square which is mounted in D.P.X. or Eukitt between 2 large glass slides (76 mm × 50 mm).

Such a colored filter is specific a) of the stain used in the technic, b) of the colored intensity depending of the stainability of the structures present in the section, c) of the electric and then, the light intensity, so that all microphotographs will be performed at the same light level.

These colored filters are costless, and quickly made when a large amount of Agar medium is prepared and stocked in

advance. Colored scales can be used when different thresholds are present in the section (for example: P.A.S. reaction for muscle fibres). These filters can also be employed with automatic image analysors for selecting the different areas to quantify.

- 1 Acknowledgments. The authors wish to thank Dr O.G. Gaudin of the Bacteriology Laboratory for her contribution in preparing the medium, and also Mrs A. Pontier for her technical help.

## Porcine skeletal muscle for physiological studies<sup>1</sup>

Esther M. Gallant

*Department of Veterinary Anatomy, Pharmacology and Physiology, Iowa State University, Ames (Iowa 50011, USA), 11 August 1978*

**Summary.** The common digital extensor (lateral head) is one of the few porcine muscles of convenient dimensions for in vitro studies of intact cells. Its removal and use in physiological studies are described.

Muscles from domestic mammals, such as the pig, are useful for physiological studies in that porcine muscle diseases often seem similar to those of man (i.e. malignant hyperthermia). In this regard, studies of domestic mammals may at times be more relevant to man than those using rodents. Previously, physiological investigations of porcine muscle have been limited by the difficulty in obtaining suitable intact cell preparations. Recently, however, I have used small bundles of intact muscle cells from the lateral head of the common digital extensor (CDE) for a study of the mechanical and electrical properties associated with porcine malignant hyperthermia. This muscle also could prove valuable for other studies of porcine muscle.

**Methods.** The porcine CDE consists of 3 distinct parts which lie on the cranialateral aspect of the antebrachium. The slender lateral-CDE arises from the proximal lateral radius by a short broad tendon and inserts indirectly on the 4th as well as the 5th digit by relatively long tendons (figure). The muscle was removed via a midlateral incision, identifying the distal tendon and dissecting to the proximal tendon. The fusiform muscle varies in length from about 5 cm in a 30-kg pig to 7 cm in a full grown adult (150 kg) and has a diameter of from 0.7 to 1.0 cm. The lateral-CDE must be further subdivided for adequate oxygenation during physiological experiments. The cells run approximately parallel to its long axis, and the muscle can further be subdivided into 2–6 bundles (3 × 0.2 × 0.4 cm or smaller) of intact cells attached to a tendon at both ends.

For histochemical studies, a transverse segment of the muscle was dipped in talcum powder and frozen in liquid nitrogen. Unfixed serial cryostat sections were stained for alkali stable ATPase<sup>2</sup> and NADH-diaphorase<sup>3</sup>. Fibre types were determined for 2–3 bundles from 6 different areas of the muscle cross-section, and the counts were pooled.

For physiological studies, muscles were stored at ambient temperature in pig physiological salt solution (NaCl 114 mM, KCl 4 mM, CaCl<sub>2</sub> 2.35 mM, MgCl<sub>2</sub> 0.85 mM, NaH<sub>2</sub>PO<sub>4</sub> 1 mM, NaHCO<sub>3</sub> 15 mM, glucose 11.1 mM, pH 7.3–7.4) bubbled with 95% O<sub>2</sub>/ 5% CO<sub>2</sub>. Isometric tension was measured using a Grass stain guage, and muscles were stimulated with supramaximal 1-msec pulses via platinum plate electrodes running parallel to the fibres.



The skin and fascia have been removed from lateral surface of a pig thoracic limb to expose the muscles: 1 extensor carpi radialis; 2 abductor digiti I longus; 3, 4, 6 common digital extensor, medial, middle and lateral heads; 5 extensor digiti II; 7, 8 lateral digital extensor, medial and lateral heads; 9 ulnaris lateralis (s. extensor carpi ulnaris). The lateral head of the common digital extensor (6) can be removed with both tendons intact. Drawing by Peter Callahan.

**Results.** Although the muscles of the distal limb are often variable in occurrence, the lateral-CDE was present in both limbs of all but 1 of more than 50 pigs (mostly Poland Chinas) which were examined. Histochemical analysis showed a muscle of mixed fibre composition with 26% type I and 74% type II fibres as identified by the alkaline ATPase reaction. Type I fibres were nearly all high in oxidative capacity by the NADH-diaphorase reaction. The type II fibres were  $\frac{1}{3}$  intermediate and  $\frac{2}{3}$  low in oxidative capacity with only a few type II fibres being classified as high oxidative.

The muscle preparation remained viable at room temperature in physiological salt solution for at least 12–16 h. In a 38 °C bath over a period of 1–2 h (the longest examined), the maximum tetanic tension remained constant. The average maximum isometric tension ( $n=26$ ) was 2.47 kg/cm<sup>2</sup> (SEM-0.1).

**Discussion.** The porcine lateral-CDE is a small muscle which can be dissected into several bundles of intact cells attached to both tendons. The muscle is composed of a mixed population of fibre types. It is intermediate in fibre composition between the porcine longissimus muscle (white)<sup>4,5</sup> and the trapezius (red)<sup>4</sup>. Physiological studies will therefore represent an average of the characteristics of the

various types as is the case with the commonly studied rat EDL and gastrocnemius muscles. (In contrast, the external intercostal is similar in fibre composition to the red porcine trapezius<sup>4</sup> and exhibits physiological characteristics (unpublished data) similar to slow twitch muscles such as the rat soleus<sup>5-7</sup>.) The lateral-CDE preparation has the advantages of easy access, small size and easy subdivision, and mixed fibre composition; and would be useful to others contemplating the study of porcine muscle physiology.

- 1 Acknowledgments. The pigs used in this investigation were provided by Drs D.G. Topel, L.L. Christian and G.A. Gronert whose cooperation is appreciated. Gratitude is also expressed to Dr N.G. Ghoshal for assistance with anatomical identifications.
- 2 H.A. Padykula and E. Herman, *J. Histochem. Cytochem.* 3, 161 (1955).
- 3 A.G.E. Pearse, in: *Histochemistry, Theoretical and Applied*, vol. II. Williams and Wilkins Co., Baltimore 1972.
- 4 W.G. Moody and R.G. Cassens, *J. Anim. Sci.* 27, 961 (1968).
- 5 A.S. Davies, *J. Anat.* 113, 213 (1972).
- 6 E. Gutmann, J. Melichna and I. Syrový, *Physiol. bohemoslov.* 23, 19 (1974).
- 7 J.J. McArdle and E.X. Albuquerque, *J. gen. Physiol.* 61, 1 (1973).

## OECOLOGICA HUMANA

### Consideration on the effects of pollution at community and population level

O. Ravera

*Department of Physical and Natural Sciences Euratom, Joint Research Center I-21020 Ispra/Varese (Italy)*

**Summary.** The effects of pollution at population level are considered in relation to demographic characteristics and overall to the birthrate. The direct and indirect effects of pollution on community structure is discussed. The influence of pollution may vary according to the food-chain structure; (as a consequence, the hazard 'ceteris paribus' will be greater in freshwater communities than in marine ones). Relations between 'diversity' and 'stability' are discussed. In addition, the advantages and difficulties in using 'diversity' and 'biotic' indices for monitoring polluted water are taken into account.

Pollution is commonly defined as the intrusion of toxic substances into a natural environment, without considering the relationship between the charge of pollutant and the self-purifying capacity of the environment. As a consequence, 'waste acceptability units' are calculated solely on the basis of pollutant concentration in the effluents.

There are, however, many substances which, although nontoxic, may profoundly alter an ecosystem (for instance phosphates, nitrates, clay, limestone powders and cement). Thermal energy can drastically modify community structure, productivity and biomass and may therefore also be considered a pollutant.

Several authors include within the terms of the definition any event, whether natural or induced by human activities, which alters the state of an ecosystem. This conflicts with the definition of pollution "sensu stricto" and it groups radioactive waste materials, heavy metals, hydrocarbons and biocides in the same category as earthquakes and volcanic eruptions. Consequently the damage done by man to his environment appears natural, and this concept can weaken the actions taken to reduce pollution.

In this work, the term 'pollution' includes any influence exerted on the ecosystem, by man or his activities, that is

strong enough to produce harmful effects at the population level and hence upon the community.

#### *Effects at population level*

For ethical reasons man protects himself against pollution at both individual and population levels. The same criterion cannot be applied to the protection of animal and plant populations, because in these cases the value of an individual is inversely related to the reproductive capacity of the population to which it belongs. For instance, the destruction of 40–60% of an algal or bacterial population does not usually jeopardise its survival, whereas the elimination of the same percentage of a low-fertility population (e.g. zebras) could severely damage or even lead to the extinction of the population.

Certain parameters are peculiar to the population, others are common to the population and individuals. Among the parameters typical of the population birth rate, spatial distribution, or density, may be mentioned. Population is not just a random group of individuals belonging to the same species and living in the same environment. It is rather a group of individuals differentiated from other