Materials and methods. In order to investigate the validity of the previous hypothesis, we chose Ubbart chickens as experimental animals, as their high sensitivity to neurotoxic substances was demonstrated. Experiments started with 49-day-old animals and were carried on for a maximum of 65 days. Chemicals were daily applied (1 g/kg b.wt/day) by a paintbrush on a shaved skin portion (ca. 10 cm^2) on the thoracic side area, gradually increasing the dose as the b.wt of the animal increased. Each mixture was tested on a group of 5 experimental animals, for a total of 5 groups (A to E, see table); a sixth group of 5 untreated animals was kept as control.

Histological and microstructure analyses were made on spinal cord cervical and lumbar swellings, as well on the sciatic nerve followings perfusion of animals with 2.5% glutaraldehyde in saline buffer (pH = 7.2) according to McEvans. Paraffin and maraglas inclusions were used.

Results and discussion. 1. Chicken from both A- and B-groups, as well as control group, never had symptoms of paralysis. However, optical and electronic microscopy showed that 10% peripheral neurites of sciatic nerve fibre bundles had been modified in treated, with contrast to control, animals. Myelinic sheath appeared unlaminated and degenerated in some parts; ball-shaped swellings were present and consequently in some cases myelinic sheath was broken. The ratio of neurofilaments to neurotubules in the axoplasm was modified, the number of the latter decreased.

2. 4 out of 5 chicken from C group had been completely paralyzed after 28, 36, 58 and 64 days, respectively; according to the length of treatment, 30 to 70% neurites showed important deterioration of myelinic sheath, which had frequent breaks, above all in largest neurites.

3. In group D also, 4 out of 5 animals were paralyzed, after 27, 45, 58 days, respectively. The microscopic analysis revealed that up to 95% neurites from both lumbar swelling and sciatic nerve had been strongly modified at 42, 56, 64 days after the beginning of the experiments: The myelinic sheath was swollen and frequently broken (figure 1); in the axoplasm neurofilaments prevailed over neurotubules; the letters tended to link up in a ring shape (a stage which preceeds degeneration); the presence of dense bodies (200–800 nm in diameter) with granular or lamellar contents of the lysosomial type was also observed (figure 2).

4. In group E chicken, 4 out of 5 animals were paralyzed after 12,, 12, 45, 65 days, respectively.

The table shows that high concentration of n-hexane and methylpentanes were with A- and B-groups, without evident signs of paralysis. On the contrary, the presence of appreciable amount of cyclohexane (D- and E-groups) match the maximum damage observed during the experiments. The known neurotoxic power of TOCP, even at low concentrations, has been confirmed by our findings. Cyclohexane is often present in glue compositions and it has always been detected in shoe factories' atmosphere[35–37]; the compound can be absorbed both by the respiratory tract and by the skin. We should like to suggest that this compound must be considered as one of the most powerful agents in causing neuropathy. TOCP also, even if present in low quantities in synthetic leathers, could induce the same dangerous effects, but most probably only by absorption through the skin.

Research is in progress in order the better to substantiate previous findings and hypotheses, also through the study of the effects of cyclohexane metabolites.

35 L. Rossi, A. Rubino and G. Tangredi, Folia med. *56*, 165 (1973).
36 A. Salvadeo, R. Colombi and R. Corsico, Lav. Umano *15*, 549 (1963).
37 A. Salvadeo, R. Colombi and G. Pezzagno, Lav. Umano *15*, 529 (1963).

Regeneration of entire legs in cockroaches as a model for developmental events

J. L. Denburg[1] and P. M. Whitington

Neurobiology Department, Research School of Biological Sciences, Australian National University, Canberra, A. C. T. (Australia), 17 May 1977

Summary. Young cockroach nymphs have the ability to generate entire legs which become the same size as normal legs when the animal is an adult. Studies of the physiological, structural and biochemical properties of some of the regenerated muscles suggest that these muscles are different from normal ones.

When the axons of identified motor neurons from the cockroach *Periplaneta americana* are cut, they regrow and eventually form functional connections exclusively with the muscles to which they were originally attached[2]. We have been studying this phenomenon with the aim of identifying the mechanisms responsible for the specificity of the interactions between motoneurons and muscles[3] and of identifying the macromolecules involved in this[4]. However, of ultimate interest to us is the ability to extrapolate from our observations of axonal regeneration in adult cockroaches to events occurring during the initial formation of neuromuscular connections in embryonic development. As yet there is no evidence that the mechanisms determining the innervation of muscles in these 2 situations are similar. Because of the difficulty in studying this process in embryos, it was decided to examine the feasibility of using the regeneration of new entire legs as a model for developmental events.

It had previously been shown that *P. americana* has the ability to regenerate entire new legs even when the old leg is removed above the level of the coxa[5]. In order to examine the physiological, anatomical and biochemical properties of the regenerated coxal depressor muscles it was necessary that the regenerate legs be nearly equal in size to normal legs when the animals have become adults. In all our studies 1 metathoracic leg was removed just above the coxa from cockroach nymphs of various ages. It was observed that the size of the regenerated leg in the adult depended on the age of the experimental nymph. The length of the nymphs was used as a measure of their age. When nymphs 15–17 mm in length were operated on, the regenerated leg in the adult was significantly smaller than the contralateral control leg (figure 1). However, when 10–11 mm nymphs or smaller were

1 Present address: Zoology Department, University of Iowa, Iowa City (Iowa 52242, USA).
2 D. Young, in: Developmental Neurobiology of Arthropods, p. 179. Ed. D. Young. Cambridge University Press, New York 1973.
3 J. L. Denburg, R. L. Seecof and G. A. Horridge, Brain Res. *125*, 213 (1977).
4 J. L. Denburg, Nature *258*, 535 (1975).
5 H. Penzlin, Wilhelm Roux Arch. EntwMech. Org. *154*, 434 (1963).

used the regenerated leg in the adult was nearly identical in size to the control leg (figure 2). 6 animals of this size represent the experimental subjects of this study.

In all the experimental animals the regenerated leg apparently functioned in a normal manner. It was used to hold on to a surface when the animal was turned upside down. It also moved in the appropriate rhythm with respect to the contralateral metathoracic leg during walking or when the animal was immobilized on its back.

The innervation of the coxal depressor muscles was investigated in 2 of the regenerate animals by electrophysiological techniques. Evidence for misinnervation was obtained in one of these; intracellular muscle recordings suggested that muscle 179 was innervated by more than 1 neuron whereas in normal animals this same muscle is innervated by a single motor neuron, D_f[6]. No signs of misinnervation were found in the other animal assayed.

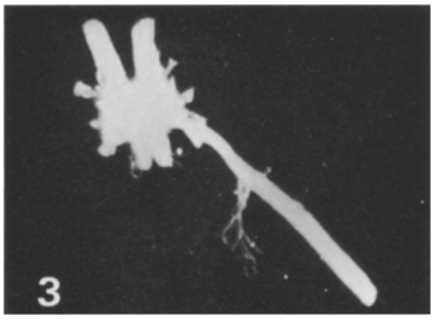
The anatomy of the coxal depressor muscles was observed to have altered in all the experimental animals. Usually 6 coxal depressor muscles can be easily identified and separated from one another. In the regenerates 4 small muscles were fused together. This prevented the identification of all of the individual coxal depressor muscles in the regenerates. In addition, the branching pattern of the nerve root containing the axons of the motor neurons that innervate the coxal muscles was completely different from that of normal animals (figures 3 and 4).

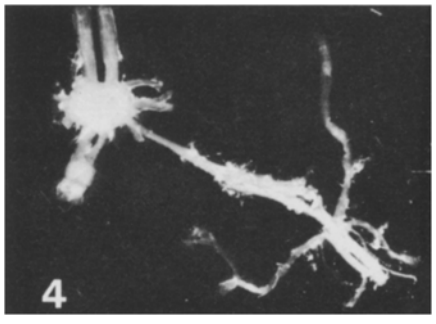
Each of the coxal depressor muscles can normally be characterized by its protein composition as determined by SDS polyacrylamide gel electrophoresis[4]. In all cases, the muscles in the regenerated leg had different gel protein patterns from muscles in control legs. In addition, in the animal in which multiple innervation of 179 was observed, the gel protein pattern of this muscle could not be distinguished from those of the other coxal muscles. In contrast, in the animal where 179 was correctly innervated the gel protein pattern of this muscle could easily be distinguished from that of the other coxal muscles. This is further evidence that the presence of some proteins used to characterize the gel protein pattern is best correlated with the innervation of the muscle by particular motor neurons.

These observations suggest that the events occurring when a cockroach nymph regenerates a new leg are different from those occurring during embryonic development and adult axonal regeneration. The variations observed in structure, innervation pattern and protein composition of the coxal depressor muscles, which were apparently still used in a normal manner, is in contrast to the precise manner in which these properties appear after embryonic development and axonal regeneration.

6 K. G. Pearson and J. F. Iles, J. exp. Biol. *54*, 215 (1971).

Figs 1 and 2. Adult cockroaches which had one of their metathoracic legs completely removed when they nymphs 15–17 mm long (figure 1) and 10–11 mm long (figure 2). Arrows indicate regenerated legs.





Figs 3 and 4. Metathoracic ganglia together with the long portion of the nerve root that contains the axons of the motor neurons that innervate the coxal muscles from a normal animal (figure 3) and one with a regenerated leg (figure 4).