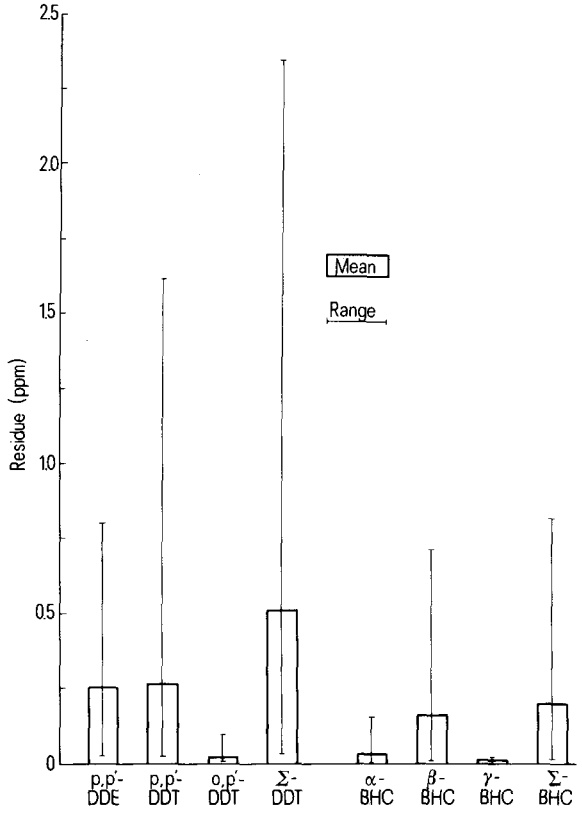
containing digested fat was discarded. The n-hexane phase was washed with distilled water until it was neutral to litmus and concentrated to a suitable volume. Residues were identified and quantified in a Packard gas-chromatograph (Model 7624) equipped with a tritium source electroncapture detector and using a glass column, 1 m by 3.2 mm packed with the mixture of 1.5% SP-2250 and 1.95% SP-2401 on 100-120 mesh Supelco-Port. Detector, column and injector temperatures were 200, 190 and 210 °C respectively. Nitrogen at 70 ml/min was used as a carrier gas. Recovery of isomers of BHC and metabolites/isomers of p,p'-DDT from the spiked samples was above 80%. The results were not corrected for recovery. In addition to gas-chromatography, the identities of the pesticides were confirmed by TLC using silver nitrate-incorporated alumina[9]. The fat content of the milk was estimated by following the method described by Polishuk et al.[3] and ranged from 0.72 to 4.98% with a mean level of 2.54.



Mean and range of DDT and BHC residues in human milk samples, in India.

Results and discussion. DDT and BHC residues were present in all 75 samples of human milk. The range and mean levels of residues on a whole milk basis are depicted in the figure. DDT residues mainly occurred as p,p'-DDE and p,p'-DDT, while minor amounts of o,p'-DDT were also detected. The major part of the BHC residues consisted of the β-isomer along with small quantitiejs of α- and γ-isomers. DDT-residues (εDDT) ranged from 0.04 to 2.25 ppm and that of ε-BHC ranged from 0.014 to 0.820 ppm. Residue values when expressed on a fat basis ranged from 1.4 to 102.2 ppm in the case of εDDT and from 1.25 to 27.52 ppm in the case of εBHC.

The mean level of DDT-residues (0.51 ppm) found in human milk in India was more than the level reported from USA, Canada, Europe and Australia[1-2,10], though, the highest concentration (4.07 ppm) of DDT in human milk has been observed in Guatemala[11]. BHC residues in human milk in India have also been found to be higher than those reported from most of the countries of the world, except Japan[12]. DDT present at an average level of 0.5 ppm in milk represents an infant intake of 0.09 mg/kg/day, which is 18 times the acceptable daily intake (0.005 mg/kg/day) recommended by the WHO[13]. As no acceptable daily intake for BHC has been established, it is difficult to assess its potential hazards. However, these results as well as the earlier investigations[5-8] suggest the need for the reduction of body burdens of these insecticides.

1 This research has been financed in part by a grant made by the United States Department of Agriculture, Agricultural Research Service authorized by Public Law 480.
2 M. Wassermann, L. Tomatis and D. Wassermann, Pure appl. Chem. *42*, 189 (1975).
3 Z.W. Polishuk, M. Ron, M. Wassermann, S. Cucos, D. Wassermann and C. Lemesch, Pestic. Monit. J. *10*, 121 (1977).
4 G. Lofroth, New Sci. *40*, 567 (1968).
5 W.E. Dale, M.F. Copeland and W.J. Hayes, Jr, Bull. Wld Hlth Org. *33*, 471 (1965).
6 M. Ramachandran, M.I.D. Sharma, S.C. Sharma, P.S. Mathur, A.G. Arvindakshan and G.C. Edward, J. Commun. Dis. *6*, 256 (1974).
7 R.L. Kalra and R.P. Chawla, Symp. Land and Water Management in Indus-Basin. India 1979.
8 H.C. Aggarwal, M.K.K. Pillai, D.V. Yadav, K.B. Menon and R.K. Gupta, Bull. Wld Hlth Org. *54*, 3526 (1976).
9 G.S. Dhaliwal and R.L. Kalra, Pestic. Monit. J. *12*, 91 (1978).
10 C.I. Stacey and B.W. Thomas, Pestic. Monit. J. *9*, 64 (1975).
11 A.E. Olszyna-Marzys, M. de Campos, M.T. Farvar and M. Thomas, Boll. sanit. Panam. *74*, 93 (1973).
12 S. Matsushima, J. clin. Nutr. *40*, 555 (1972).
13 Wld Hlth Org. Tech. Rep. Series No. 502 (1972).

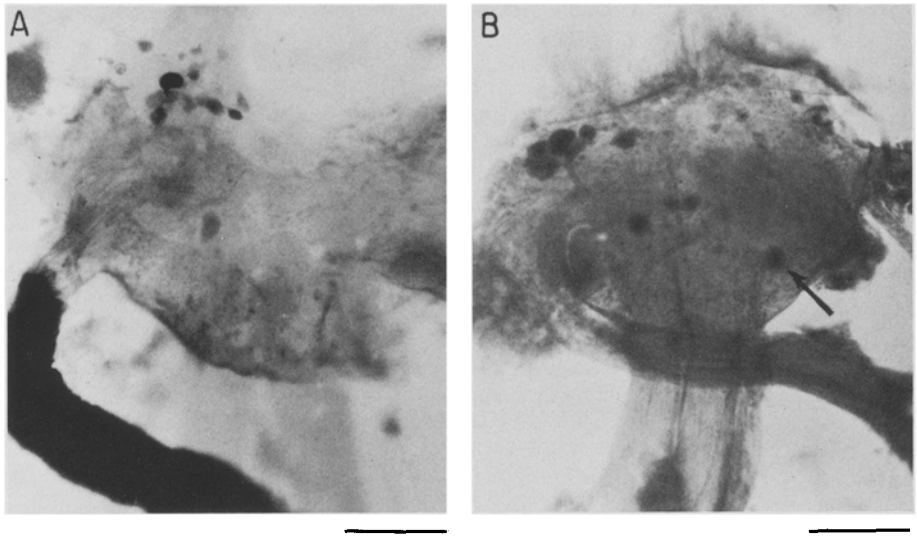
Anatomical evidence for cross regeneration of motor axons in a cockroach[1]

V. Krauthamer[2] and C.R. Fourtner

Department of Biological Sciences, State University of New York at Buffalo, Buffalo (N.Y. 14260, USA), 28 July 1980

Summary. The technique of electrophoretic application of intracellular markers is used to demonstrate that motor axons can regenerate into the contralateral limb of a cockroach after the nerve has been crossed to the contralateral side.

Recent studies have demonstrated that regeneration of neural elements in the cockroach occurs when the nerve roots of thoracic ganglia are manipulated so that their processes grow into the contralateral limb. By rotating the mesothoracic ganglion 180° and using a cobalt filling technique, Bate demonstrated that axonal processes of regenerating motor neurons could penetrate their original contralateral limb[3]. More recently, Fournter et al. developed a procedure by which the proximal stump of one metathoracic leg nerve (right nerve 5) was crossed to the



Photomicrograph of the metathoracic ganglia in cross-regenerate organisms. *A* Ventral view of a ganglion stained with CoS. Note the heavily stained nerve (5) crossing from the left to the right side and the population of cells in the anterior, contralateral region. *B* Ventral view of metathoracic ganglion filled with Lucifer yellow. This is a negative photograph again showing contralaterally filled motor neuronal somata. In only this preparation did we see an ipsilateral soma filled. Bar = 200 nm.

opposite side and placed end-to-end with the distal segment of its contralateral homologue (left nerve 5)[4]. After 3–4 weeks functional regeneration of the trochantoral hair-plate-to-femoral-extensor reflex occurred; the reflex now consisting of the peripherally originating left side afferents and a centrally originating right motor neuron (D_s). However, using a Co^{++} backfilling procedure, they were unable to demonstrate a direct anatomical continuity between the distal segment of nerve 5 and the motor elements in the right hemiganglion. In the region where the ends of the nerves ajoin, a substantial scar tissue developed and Co^{++} never progressed through this region. This inability of cobalt to traverse this region did not permit direct anatomical observations confirming the physiological evidence that contralateral regeneration occurred. Using intracellular iontophoresis of Co^{++}, Fourtner et al. demonstrated anatomical evidence that the cell body of one motor neuron, D_s, was contralateral with respect to the muscle it innervated. However, no evidence was available to: a) demonstrate the number of motor neurons which may have regenerated and grown into the contralateral limb; and b) that only those motor neurons contralateral to the reinnervated limb regenerated via the crossed nerve 5. Therefore, we have re-examined the cross regenerates using a more thorough experimental procedure to investigate the regeneration of motor axons to the left leg in the cross regenerate organisms.

The surgical procedures for producing the crossed-regenerates and the testing paradigm for successful regeneration have been described previously[4]. After the regenerating animals had developed positive reflexive tests, they were pinned ventral surface upward and nerve 5 in the left leg was exposed in either the femur or the distal third of the coxa. A short section of the nerve was dissected free and cut; the proximal stump was placed in either a small pool or a polythylene tube of backfilling solution. Two solutions, 10% $CoCl_2$[5] and 4% Lucifer yellow-CH[6], were used. The solutions were electrophoresed into the cut axons using direct current.

Electrophoresis for periods of less than 8 h did not produce any stain past the scarred region. However, if a constant current of 100 nA was applied for 24 h, both the cobalt and the Lucifer yellow traversed the scarred region and filled cell bodies in the right hemiganglion. In 5 preparations (2 with Co^{++} and 3 with Lucifer yellow) contralateral cell bodies were filled with marker. In 1 case there was a single cell body ipsilateral to the limb innervated; this anomalous result may be due to an inadvertant backfill of a motor axon from an ipsilateral nerve. The figure illustrates an example of successful experiments using Co^{++} and Lucifer yellows. The cell bodies filled with the marker are located in the region expected for motor neurons innervating the distal segments of the leg such as the fast flexors of the tarsus and pre-tarsus[7]. There were at least ten somata filled in each of the 5 preparations.

It was necessary to electrophoretically drive the markers for much longer periods of time compared to normal passive backfilling (i.e. applied electrical current) which occurs within 4–8 h. The slow movement of cobalt and Lucifer yellow through the scarred region may be due to the small size of the neural processes in this region. This conclusion is supported by physiological data in which the compound afferent action potential is much wider in the regenerate organisms and preliminary ultrastructural studies of the scarred regions which demonstrate that axons in this region are much smaller in diameter than axons of nerve 5 from a normal animal (Fourtner, unpublished).

These data support the hypothesis that in cross-regenerating organisms the motor axons grow through the scar tissue and directly innervate the muscles of the limb and that the cell bodies of these regenerating motor axons are localized in the contralateral hemiganglion. Furthermore, these data support the previous physiological studies that demonstrated the reflexes formed in the cross regenerate organisms are from afferents from the right limb and motor axons whose cell bodies are in the left hemiganglion.

1 Supported by NIH grants IRO1NS1420401 and 5KO4NS0014102.
2 Current address: Department of Physiology, New York Medical College, Valhalla, N.Y. 10975.
3 C.M. Bate, Experientia *33*, 451 (1976).
4 C.R. Fourtner, C.D. Drewes and T. Holzmann, J. Neurophysiol. *41*, 885 (1978).
5 K.G. Pearson and C.R. Fourtner, Can. J. Zool. *51*, 589 (1973).
6 W.W. Stewart, Cell *14*, 741 (1978). Kindly provided the Lucifer yellow.
7 C.R. Fourtner and K.G. Pearson, in: Identified Neurons and Behavior of Arthropods, p.87. Ed. G. Hoyle. Plenum Press, New York 1977.