Studies on the etiology of the experimental neuropathy from industrial adhesive (glues)

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Summary. Chickens treated by paintbrushing with glue distillate (used in shoe industries), hydrocarbon mixture and TOCP in hexane, developed paralysis ($^4/_5$); in these, we have demonstrated a diffuse degeneration of the myelinic sheath of the peripheral and central neurites. Besides TOCP, cyclohexane (because of its higher concentration in the glue distillate and in the hydrocarbon mixture) may be indicated as responsible for occupational neuropathy.

Several years ago a hitherto unknown occupational neuropathy occurring among workers in shoe factories was observed[5]. The onset of the occupational disease is deceptive and aspecific: it is accompanied by anoressia, nausea, epigastralgy, vomit, headache, drowsiness or sleepiness and always by loss of weight. On the basis of some more peculiar features and of a different development, we know a 'slight' and a 'serious' polyneuropathy. The 'slight' polyneuropathy is characterized by muscular tiredness, paresthesias, pain, muscle cramps that symmetrically involve arms and legs, but preferentially and more significantly the latter. The 'serious' polyneuropathy presents a very short prodromic period with a rapid development to the tetraparesis even after interruption of occupation. Some months after the flaccid paralysis occurred, a slow process of improvement with a duration of 1–2 years takes place, leading to restoration of motory function but not to a complete normalisation of the electromyographic symptoms.

Actually there are a 3 hypothesis about chemical agents possibly responsible for this occupational disease:

1. tri-o-Cresylphosphate (TOCP): High boiling compound often present as a plasticizer in synthetic leather. Discordant findings emerge from the literature about the synthetic leather TOCP concentrations: This is due to the difficulty of a analytical detection of the product. Recently, by using a chromatographic specific method, the TCP has been detected at different concentrations, from 0.1 to 3% (average 1.1%), within about half the synthetic leathers considered[6,7]. TOCP and TCP has been demonstrated to induce neuropathy if taken orally at low doses[8–23].

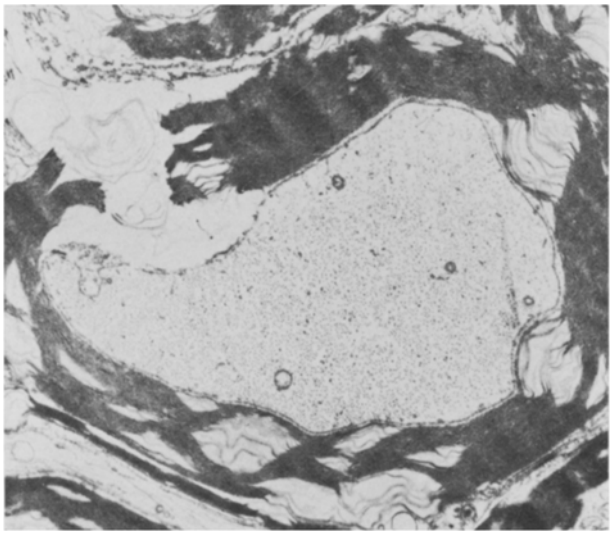

Fig. 1. Glue distillate treated chicken. Central neuronal damage of the lumbar swelling. The picture demonstrates the grave damage of the myelinic sheath. ×12,000.

2. n-Hexane: Low boiling hydrocarbon usually present as a component in solvent mixtures used in the manufacture of glues for leather and shoe industries[24–31].

3. Combined action by different volatile compounds present in glue solvents, of which n-hexane would not be a major factor in causing neuropathy[31–34].

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Composition of solvent mixtures tested on various chicken groups[a]

Group Solution used	A Hexane solvent[b]	B Leather extract[c]	C 2% TCP in hexane ethylacetate[b]	D Glue distillate[d]	E C_4–C_8 hydrocarbon mixture[e]
2-Methylbutane	0.7	0.6	0.6	0.19	3.2
n-Pentane	3.3	2.5	2.4	0.88	5.3
Cyclopentane	0.8	0.6	0.6	0.18	1.7
2-Methylpentane	19.3	14.5	14.2	11.90	10.6
3-Methylpentane	18.7	14.0	13.8	11.60	6.9
n-Hexane[b]	35.2	26,4	25.9	16.60	8.8
Methylcyclopentane	19.0	14.2	13.9	9.80	12.4
Cyclohexane	3.1	2.2	2.1	17.10	14.1
Ethyl acetate	–	25.0	24.5	7.80	–
2-Butanone	–	–	–	15.80	–
Benzene	–	–	–	2.55	–
Trichloroethylene	–	–	–	2.55	–
Toluene	–	–	–	3.05	–
C_7–C_8 hydrocarbons	–	–	–	–	37.0
TCP	–	–	2.0	–	–

[a] Determined by gas-chromatography; values in percent weight. [b] Hexane is mixture of C_6-hydrocarbons; n-hexane is the pure straight-chain compound. [c] Obtained by refluxing thin leather strips in 3:1 hexane/EtAc. 8 different leather samples were collected at various shoe factories in Marche region (Italy) where polyneuropathy cases were recently noticed. [d] Obtained by condensing the volatile components present in 11 various glues of different makes, collected in the same factories as above. [e] 1:1-mixture of petroleum ether (40–70 °C) and ligroin (70–120 °C).

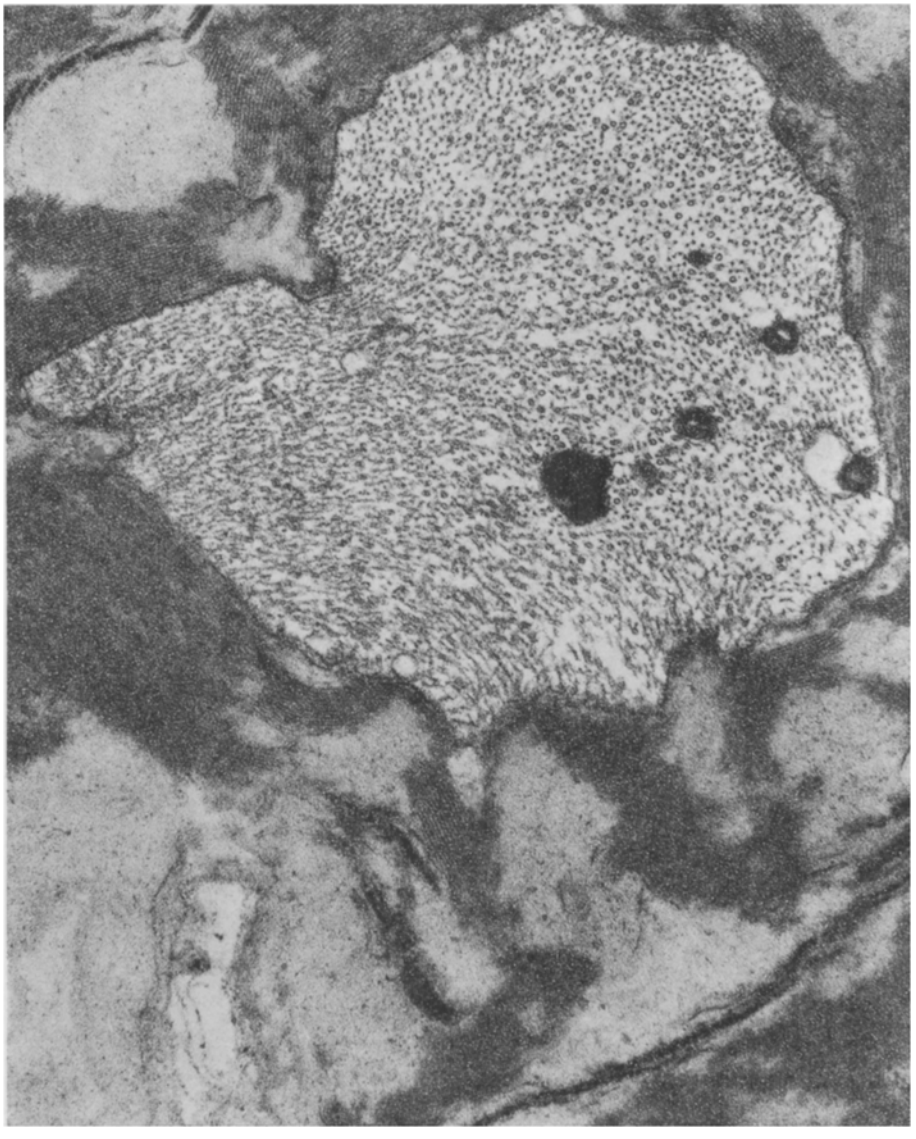

Fig. 2. Chicken treated with a glue distillate. Peripheral neuronal damage (sciatic nerve) with alteration of the myelinic sheath and lysosomes (or dense bodies) in the axoplasma. × 50,000.

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.

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

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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

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