NEUROMYOPATHY IN THE MOUSE PRODUCED BY THE ANTIMICROBIAL AGENT NITROXOLINE

FRANCIS O'GRADY AND BARBARA SMITH
Departments of Bacteriology and Pathology, St Bartholomew's Hospital, London

PLATES XV–XVII

NITROXOLINE (5-nitro-8-hydroxyquinoline) is marketed under the name "Nibiol" as a urinary antiseptic. Its antibacterial range is wide, including *Pseudomonas pyocyanea*, and it is bactericidal (fig. 1). These properties make it potentially a valuable agent, although the other compounds in this class are known to be largely conjugated before excretion (Smith, 1953; Smith and Williams, 1954), and the conjugates are unlikely to possess much antibacterial activity (Albert *et al.*, 1947).

Like the parent substance, 8-hydroxyquinoline, and some other substituted derivatives, nitroxoline is a potent antifungal agent *in vitro* (fig. 1). As part of a search for therapeutic agents for systemic mycoses, nitroxoline was tested for its ability to control an experimental candida lesion in the mouse (Thompson and O'Grady, 1959). In doses up to, and including, the LD_{50} it proved to be ineffective (fig. 2). In addition, animals receiving the large doses dragged their hind legs and had difficulty in moving about. Further investigation showed that neurological disturbances could be produced by large doses of nitroxoline given parenterally and this paper will describe the clinical picture and the pathological changes produced.

MATERIALS AND METHODS

Administration and dosage. A 0·5 per cent. solution of nitroxoline in arachis oil was injected intraperitoneally. Preliminary experiments established that the LD_{50} for albino mice weighing between 28 and 34 g. was 140 mg. per kg. body weight. Survivors were found to tolerate larger amounts, and in various experiments were treated with increasing doses up to 480 mg. per kg. at intervals of 1–16 days. Acute neurological damage developed in animals given a sublethal dose (110–130 mg. per kg.) followed 24 hr later by a dose of 200–230 mg. per kg. Chronic lesions developed in animals given 4–9 doses, each of which was not far below the LD_{50}.

RESULTS

Neurological findings

The neurological deficit was clinically mainly of two types, although mixed pictures sometimes developed. *Acutely affected* animals became drowsy about 10 min. after the injection, but did not become unconscious and would move about when stimulated. After a period of

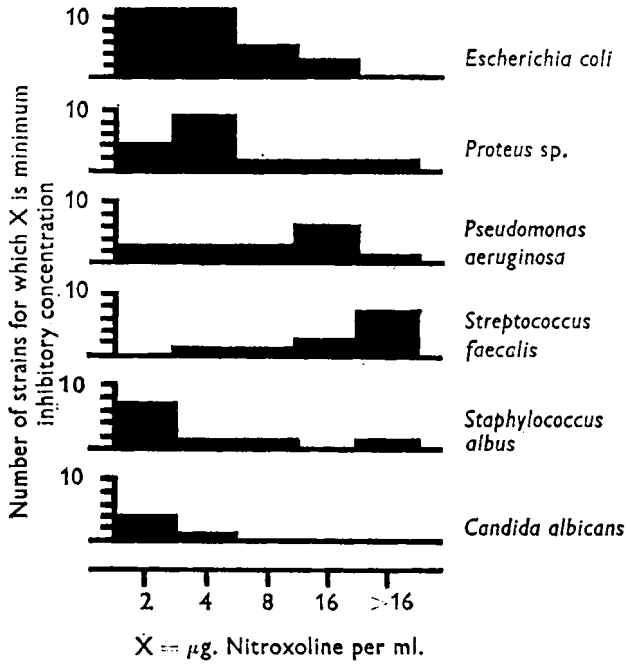

FIG. 1.—Distribution of minimum inhibitory concentrations of nitroxoline for 157 strains of common urinary pathogens and for 6 strains of *Candida albicans*.

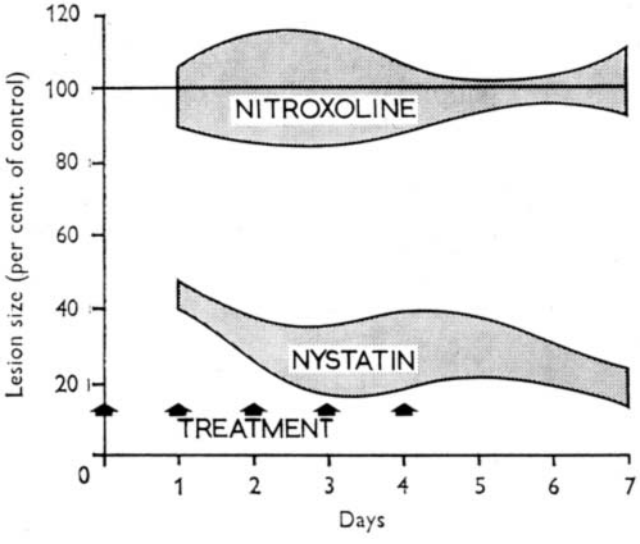

FIG. 2.—Comparison of nitroxoline and nystatin in controlling mouse thigh candidiasis. The average size of the thighs in treated animals is expressed as a percentage of the average thigh size in untreated animals. The shaded areas show the range of results in different experiments.

NITROXOLINE AND NEUROMYOPATHY

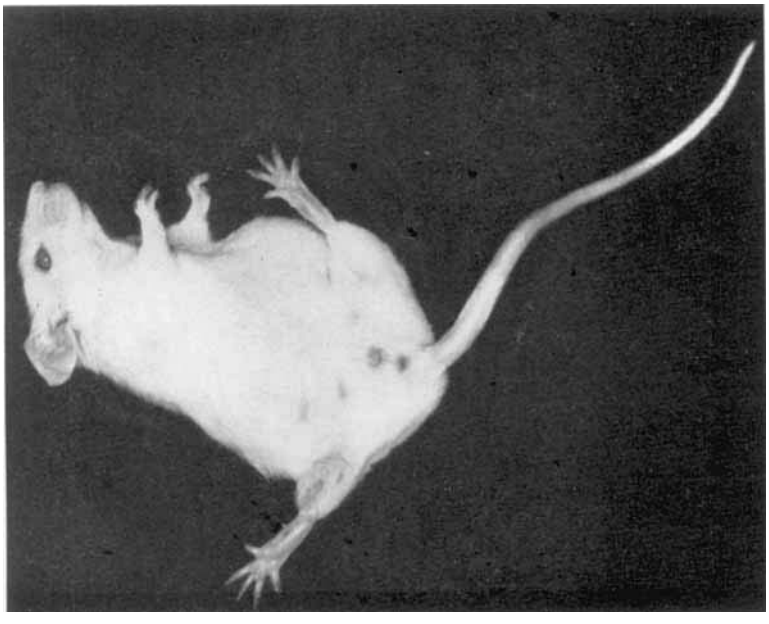

FIG. 3.—Mouse after seven injections of nitroxoline. Loss of righting reflex.

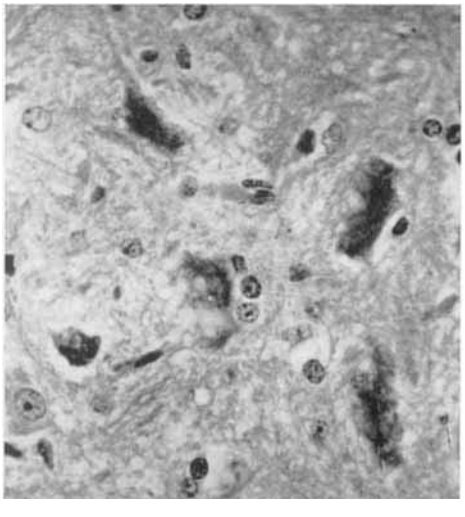

FIG. 4.—Anterior horn of quadriplegic mouse that had received two injections of nitroxoline. The neurones are poorly defined and no intracellular structure can be seen. Haematoxylin and eosin. ×360.

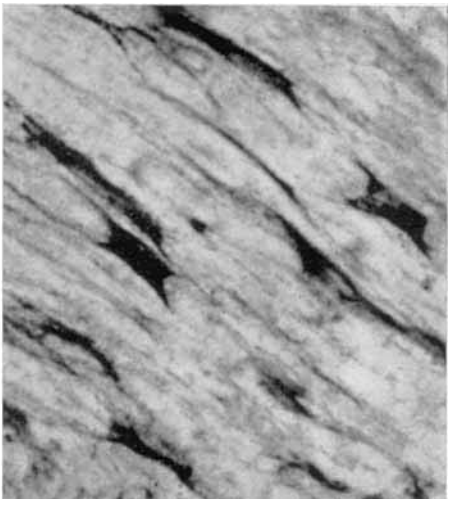

FIG. 5.—DPN diaphorase preparation of a posterior root from an animal that had received two doses of nitroxoline. The myelin sheaths are retracting away from the nodes of Ranvier. Increased enzyme activity in Schwann cell cytoplasm. ×540.

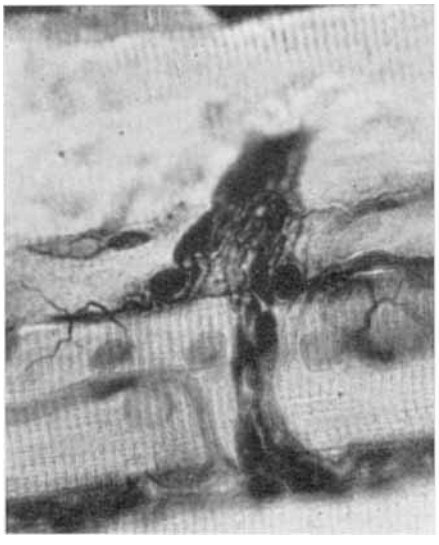

FIG. 6.—Normal motor end-plate from an affected mouse. Glees-Marsland. ×500.

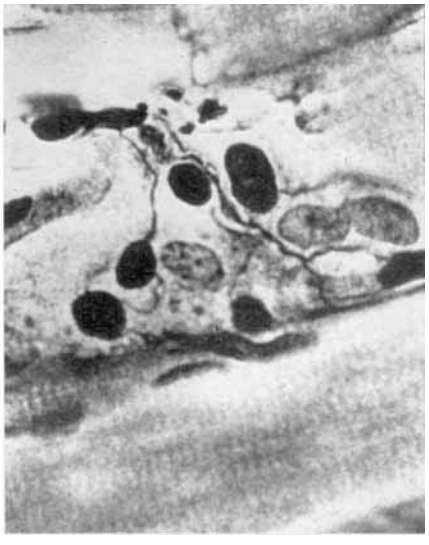

FIG. 7.—Similar preparation from an animal that received exactly the same dosage as that to which fig. 6 refers. There are argyrophil swellings on the tips of terminal axons. Glees-Marsland. ×500.

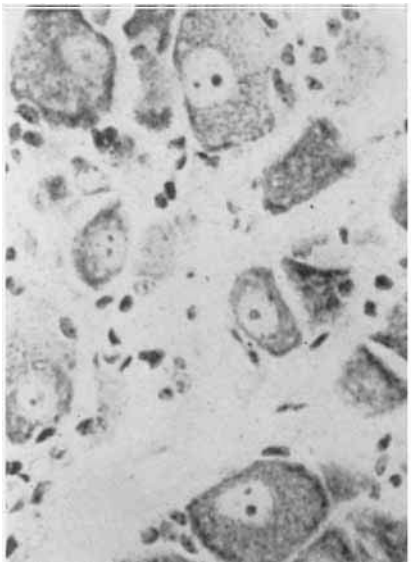

FIG. 8.—Posterior root ganglion of a mouse that had received six doses of nitroxoline, the last 11 wk before death. Some of the nuclei are eccentric and the ribosomes are displaced towards the cell membrane. Cresyl violet. ×600.

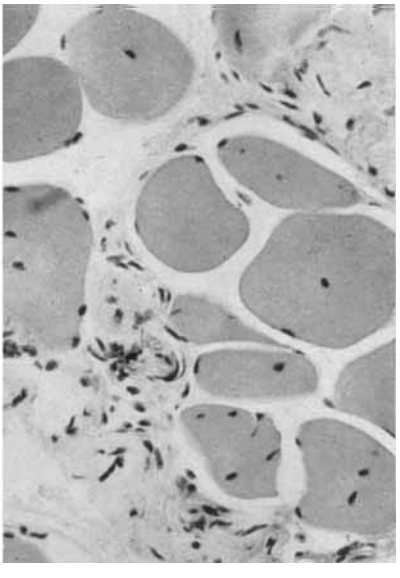

FIG. 9.—Myopathic muscle fibres from a mouse that had received seven doses of nitroxoline the last 3 wk before death. HE. ×200.

NITROXOLINE AND NEUROMYOPATHY

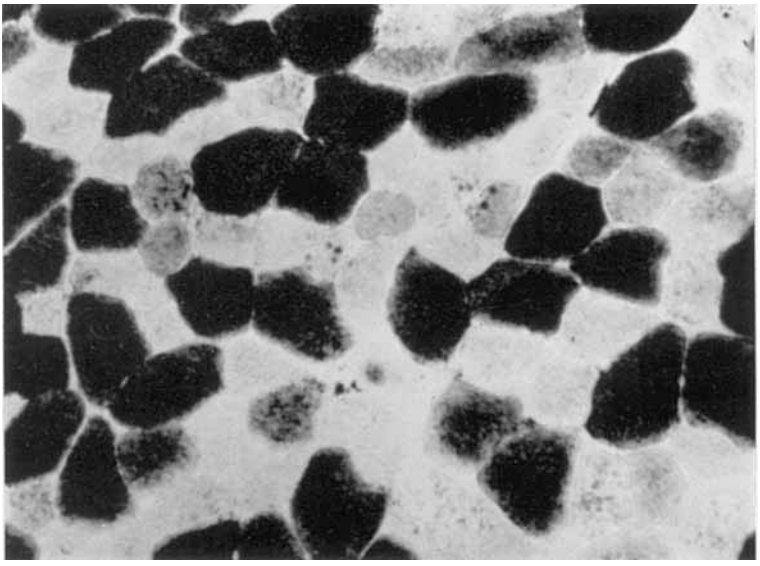

FIG. 10.—Phosphorylase preparation of the muscle of a mouse that had received six doses of nitroxoline, the last 8 wk before death. Many of the fibres contain no enzyme. ×600.

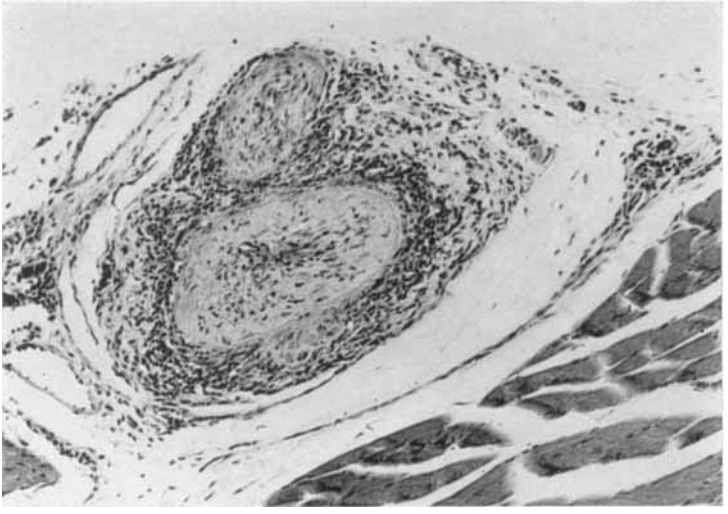

FIG. 11.—Small artery and vein from a mouse that had received six doses of nitroxoline the last 10 wk before death. The walls of both vessels show inflammatory changes and there is a cuff of lymphocytes. HE. ×105.

some hours they became quadriplegic with no movement at all in their hind legs and only a little in their fore-legs. When stimulated, they responded by a total body reflex contraction. Even when hand fed and given intraperitoneal glucose saline these animals did not survive more than two days; they probably died from liver failure. The picture was thus one of acute spastic paralysis.

The *chronically affected* animals showed less response to the injections in the early stages, but the following morning were found to be moving about slowly and with some difficulty, dragging their abducted hind legs. The tone in their limbs was variable, usually being somewhat increased, and when suspended by their tails some animals held all four limbs in acute flexion, instead of the usual extension. When the animals were placed on a wire grid inclined at 45° to the horizontal they had great difficulty in moving about, partly because of weakness but more particularly because they did not appear to know where their feet were and could not place them accurately on the wires. They waved their feet aimlessly in space, occasionally losing their balance and falling off. When they held a wire they gripped by bending a leg round it rather than putting a pad on it. When moving on a flat surface they put their feet down rather gingerly, sometimes even walking on the sides of their feet as if their pads were sore. In contrast to normal mice they could easily be turned on their backs, when they had great difficulty in righting themselves and lay waving their legs in the air (fig. 3). The general condition of the animals remained good. They were able to feed and groom themselves and were kept up to 5 mth. In long-surviving animals, once the neurological picture was established, it did not alter significantly.

Pathological findings

The animals were killed between two days and three months after the onset of symptoms, 34 altogether being examined pathologically.

Paraffin sections of heart, kidney, liver, intestine, brain, spinal cord, posterior root ganglion and skeletal muscle were stained with haematoxylin and eosin; a few were stained with cresyl violet. Frozen sections of spinal cord, brain and posterior root ganglia were stained with silver-luxol fast blue and sudan black. The skeletal muscle was stained for fat and impregnated with gold or silver. Cryostat sections of the posterior root ganglia, cord and muscle were examined for a number of oxidative and hydrolytic enzymes: the most useful proved to be diphosphopyridine nucleotide (DPN) diaphorase (cytochrome-*c* reductase) (Nachlas, Walker and Seligman, 1958), and phosphorylase (Takeuchi and Kuriaki, 1955), and only these will be reported as the others did not contribute any further information.

Acute changes

Owing to short survival of these animals the pathological changes in them were only slight. Some of the large neurones of the brain and spinal cord show irregular shrinkage with loss of definition of cell outline and poor staining compared with the normal (fig. 4). The cells

in the posterior root ganglia show early loss of Nissl substance and an occasional eccentric nucleus. It is too early to demonstrate myelin breakdown, but an occasional retraction ball is present in the posterior root, and sections incubated for DPN diaphorase show an increase in Schwann cell activity (fig. 5). The distal ends of the motor axons show considerable irregularity, and comparison with the normal suggests that some end-plate innervation may have disappeared. The other parenchymatous organs also show early signs of damage. The cardiac and skeletal muscle contain a number of swollen and unhealthy fibres, the cells of the renal tubules are poorly defined and stain weakly, although they are not frankly necrotic, and the liver which was macroscopically yellow, blotchy and soft, shows considerable fatty infiltration.

Chronic changes

Animals that had developed weakness and ataxia 3 days to 5 mth previously were examined. No abnormalities are demonstrable in the heart, lungs, brain or spinal cord and the kidneys show only an occasional collection of chronic inflammatory cells. The livers show small areas of centrilobular necrosis, the necrotic cells being replaced by collections of lymphocytes, plasma cells and eosinophils. All the animals have considerable lymphadenopathy, but histologically the lymph-glands show only severe reactive hyperplasia. The main pathological changes are in the peripheral nervous system and the skeletal muscle; the anterior horn cells, the nerve roots and the trunks are normal as are most of the motor end-plates (fig. 6). However, of the 13 animals on which silver impregnations were done two show argyrophil swellings on the tips of the terminal axons (fig. 7); no sprouting of axons was seen. The sensory root ganglia contain a number of neurones that are swollen and in which the nucleus and ribosomes are displaced to lie against the cell membrane (fig. 8). There is some increase in DPN diaphorase activity in the amphicytes and capillaries around the ganglion cells. No changes can be seen in the sensory endings in the muscle or the skin.

The changes in the muscle in standard preparations are patchy and, in most cases, not very severe. In all the animals examined, occasional groups of myopathic fibres can be found: they are swollen and have central nuclei (fig. 9), but there is very little fibre necrosis. Phosphorylase preparations show that a considerable proportion of the fibres have lost their activity (fig. 10) and appear yellow in the section, but the structure of the myofibrils and sarcolemma can still be seen. This is in contrast with cortisone myopathy where a similar loss of activity is associated with obliteration of the fibre structure (Smith, 1964). In one animal only, the blood vessels in the muscle show complete necrosis of the wall and invasion by inflammatory cells (fig. 11).

Discussion

The failure of nitroxoline to influence an experimental candida lesion, despite its antifungal activity *in vitro* is disappointing but not altogether unexpected. The disparity between in-vitro and in-vivo activity of the parent 8-hydroxyquinoline is already well known. Despite the advantage of better absorption claimed for nitroxoline, it is likely that it suffers similar prompt detoxication and inactivation. Beckett and Smith (1956) have suggested that 8-hydroxyquinoline loses its antimicrobial effect *in vivo* not from blockage of its chelating effect by conjugation (Albert *et al.*, 1947) but from some other modification of its molecule effected by contact with intact red cells. Whatever the mechanism, it appears that compounds of this class are short-lived in the body in an active antimicrobial form.

No antifungal effect was seen even when the animals were treated with near-lethal doses, but it must be emphasised that only animals given very large doses showed abnormalities, the neurotoxic doses being weight for weight at least a hundred times those suggested for man. Because of the difference in routes of administration the disparity in blood levels of the drug between mice and man was probably even greater.

The short-lived spastic animals showed evidence of damage both inside and outside the nervous system. It seems probable that nitroxoline in this dosage is a parenchymatous poison that affects the nervous system particularly severely.

The striking feature about the chronically affected animals was that the changes seen in conventionally stained preparations were relatively slight compared with the severe clinical disability. The peripheral nervous system was well visualised in silver impregnations as well as by other methods and the minor changes in the sensory neurones and motor end-plates seem inadequate to account for the ataxia, particularly in view of the fact that the spindles were probably normal. However, a four-legged animal, which cannot see its feet, may be more disabled by a partial loss of joint sense than a two-legged animal, which can.

The changes in the muscle fibres are difficult to assess: all the animals showed some evidence of myopathy, but in no case was it severe. Even in the animal that had polyarteritis most of the muscle fibres were normal histologically. The loss of phosphorylase activity in many muscle fibres could be a non-specific effect of any muscle damage (Smith, 1965), but it does suggest that this damage may be more severe than would appear from the paraffin sections. It is also possible that the loss of this particular enzyme activity, while the mitochondrial enzyme activity remains normal, may have a specific significance. Inhibition of muscle phosphorylase by nitroxoline might account for the clinical picture, but a serious obstacle to this explanation is the persistence of the disability for months after the last injection. The known metabolism of hydroxyquinolines and the failure of nitroxoline

to influence the experimental candida lesion suggest that the drug is rapidly inactivated. This makes it difficult to believe that a direct action of the drug or its metabolites could be responsible for prolonged suppression of phosphorylase activity. It suggests that nitroxoline may initiate some self-perpetuating process.

The need for several doses over 5–21 days in order to produce a lesion suggests that this might have an immunological basis. Reference to other conditions of obscure aetiology cannot lend much support to this slender hypothesis, but one animal showed arteritis—which is thought in many cases to be related to hypersensitivity—and in the human myopathy of polyarteritis nodosa the histological changes are often very similar to those seen in these mice, and the clinical incapacity is also greater than one would expect from the pathological picture. Both in human polyarteritis and in serum sickness, where the immunological basis is firmer, sensory neuropathy sometimes occurs, which is also reminiscent of nitroxoline toxicity in the mouse.

SUMMARY

It has been confirmed that nitroxoline has a wide antimicrobial spectrum *in vitro* including *Pseudomonas pyocyanea* and *Candida albicans*.

In maximum tolerated doses nitroxoline failed to influence the course of an experimental candida lesion in the mouse.

Some animals receiving several near-lethal doses developed permanent weakness and ataxia. Histologically there was a mild neuropathy and a rather more marked myopathy. Possible mechanisms for their production are discussed.

The work was aided by a grant from the Muscular Dystrophy Group of Great Britain. We are grateful to Dale Pharmaceuticals Ltd for supplying nitroxoline and to Dr Sabina Strich for her criticism. The photography was done by Mr Peter Crocker.

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