FURTHER EXPERIMENTS IN THE STUDY OF IMMERSION FOOT

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Introduction

In a previous paper a histological study was made of the changes which occur in the tail of the rat after periods of exposure to cold and wet. The periods and conditions of exposure were comparable with those under which the less severe forms of immersion foot develop in man. It was found that conspicuous damage to the muscle and nerve tissues was present after exposures of 48 hours and increased with longer exposures and that the skin and other tissues, including blood vessels, appeared to be much more resistant to chilling. The nerve and muscle tissue had not returned to normal in animals killed sixty days after exposure: and in rats surviving two months there was some evidence that muscle degeneration secondary to denervation was setting in. Treatment in the form of two heating-up processes was investigated. They were found to accelerate the initial reaction, but made no significant difference to the histological changes in a month's time.

In view of these findings and because of the histological abnormalities found in the extremities of human cases which have long survived exposure, it was considered advisable to continue the study in rats surviving exposure for a longer time, i.e. between 90 and 365 days, to see if there was any further restitution towards or departure from normality.

Experiments

The experiments were performed upon rats, and the tail was used as an experimental limb. The animals were exposed in a cold room (Temp. 3° to 4° C.). The cages were so arranged that cold artificial sea water trickled slowly through them but never reached a depth of more than about half an inch. The water in the cages was usually about 1° warmer than the air of the cold room. Food and water were suspended within reach from the cage roofs, and under these conditions the lower part of the hind legs and the tail of the rat were almost continuously immersed. It had been found that exposure to this wet and cold for more than 96 hours approached the fatal time for laboratory rats, and that 48 hours was the minimum of exposure required to produce definite damage to nerve and muscle. Accordingly rats exposed for 48 and 96 hours were used. Table I is a summary of the experiments and shows that they were subdivided into two series, depending upon the temperature at which the rats were allowed to recover from the exposure.
Series A.—The animals were allowed to recover slowly without any question of treatment, spending the first twenty-four hours after "rescue" in an unheated room (average temp. 13.5° C.) and then being transferred to the animal house (average temp. 18° C.).

TABLE I

| Series A. | Series C. |
|-----------|-----------|
| Slowly Warmed up after Exposure. | Warmed up in Air Incubator at 37° C. for Three Hours after Exposure. |
| Rat No. | Survival Time. | Rat No. | Survival Time. |
| 48 hours' exposure. | | 223 | 88 days |
| 224 | 92 days | 229 | 165 days—found dead |
| 225 | 90 " | 234 | 180 days |
| 232 | 180 " | 235 | 180 " |
| 233 | 180 " | ... | ... |
| 239 | 365 " | 241 | 365 days |
| 240 | 365 " | 242 | 365 " |
| 226 | 90 days | 227 | 90 days |
| 228 | 90 " | 230 | 150 days—found dead |
| 231 | 145 " | 237 | 180 days |
| 238 | 180 " | ... | ... |
| 236 | 183 " | 241 | 365 days |
| ... | ... | 242 | 365 " |
| 243 | 365 days |

Series C.—The animals after rescue were heated up in a 37° C. hot air incubator for three hours before being put in the animal house.

(In the previous set of experiments a series B was present, in which the animals were warmed up rapidly in shallow water at 29° C. for half an hour after "rescue." As there was no histological difference between the B and C series it was not further studied.)

Preparations Examined

In all rats a transverse section of the centre of the tail was embedded in celloidin and sections were stained with hematoxylin and eosin, and by Masson's tri-chrome method.

Longitudinal fillets of the soft tissues near the central point of the tail were studied in frozen sections, stained by Anderson's method for myelin and by Weddell's modification of Bielshowky's stain for axis cylinders. The fillets were taken with their mid-point 60 mm. from the tail base except in the case of rats 228, 238, 242 (55 mm.): 226, 243 (50 mm.): 241 (45 mm.): 239 (40 mm.): 240 (20 mm.).

Details of Series A

Rats were exposed to cold and wet for 48 and 96 hours and were allowed to survive for varying periods thereafter, from 90 to 365 days. After exposure they were allowed to warm up slowly in an unheated room before being put into the animal house.

Rats exposed for 48 hours.—In all rats the bone, blood vessels and cutaneous tissues were healthy. In those surviving for 92 days the picture was com-
paratively normal. There were a few muscle fibres which were unusually slender and which were hypernucleated. Some of the muscle-nerve leashes contained slightly fewer myelinated nerve fibres than normal. Motor nerve endings were visible on the muscle fibres. In rat 224 the presence of "ultraterminal" nerve fibres and nerve fibres wandering between the muscle fibres were evidence of regeneration of motor nerves. There was no significant fibrous tissue increase. The main nerves were well myelinated. In the 180-day survivors the histological picture was similar. In the 365-day survivors the picture in rat 240 was normal; in rat 239 (with a longer tail) there were focal areas of abnormality in two main muscle bundles, with diminution in muscle fibre calibre, with much endomysial fibrous tissue thickening; other tissues appeared healthy.

Rats exposed for 96 hours.—In all rats the bone, blood vessels and cutaneous tissues were healthy. In the 90-day survivors the muscle fibres were sometimes small and hypernucleated, and in rat 228 there was fibrous thickening of the endomysium (Fig. 1). The main nerves had lost about half

Fig. 1.—Rat 228, exposed for 96 hours (A series), survival time 90 days. Transverse section of tail muscle and main nerve. To show slenderness of many muscle fibres and slight fibrous thickening of the endomysium. × 85. Haematoxylin and Eosin.

Fig. 2.—Rat 236, exposed for 96 hours (A series), survival time 183 days. To show practically normal muscle fibres. × 85. Haematoxylin and Eosin.

Fig. 3.—Rat 243, exposed for 96 hours (A series), survival time 365 days. To show an almost normal picture. There is still some variation in muscle fibre calibre and muscle nuclei sometimes lie within the fibre. × 85. Haematoxylin and Eosin.

Fig. 4.—Rat 237, exposed for 96 hours (C series), survival time 180 days. To show marked fibrous thickening of the endomysium. × 85. Haematoxylin and Eosin.

Fig. 5.—Rat 228, exposed for 96 hours (A series), survival time 90 days. To show demyelination of main nerve. × 350. Anderson's myelin stain.

Fig. 6.—Rat 243, exposed for 96 hours (A series), survival time 365 days. To show remyelination of main nerve. × 350. Anderson's myelin stain.

their myelinated fibres but many unmyelinated regenerating fibres were present (Fig. 5). In the muscle nerve leashes there was often only one normal myelinated fibre present but there would be several regenerating unmyelinated or finely myelinated fibres (Figs. 7, 9). Re-innervation of muscle fibres was present, numerous motor nerve endings being visible (226). In a 145-day survivor, rat 231, the muscle damage was severe, with numerous small hypernucleated muscle fibres and endomysial fibrous thickening. Main nerves were better myelinated, but no motor nerve endings were seen. The 180-day survivor showed a much less extent of muscle damage. The 183-day survivor (Fig. 2) and the 365-day survivor (Fig. 3) were similar and nearer to normal. They showed no areas of fibrosis in the muscle. There was some variation in muscle fibre calibre, so that some were a little more slender than normal but they were well innervated by leashes full of myelinated fibres (Fig. 8). The main nerves had not quite returned to normal (Fig. 6).

DETAILS OF SERIES C

The rats were exposed for 48 hours and 96 hours and allowed to survive for 88-365 days. After exposure they were warmed up for three hours in a 37° C. hot air incubator.
**Rats exposed for 48 hours.**—In all rats the bone, blood vessels and cutaneous tissues were healthy. The picture in the 88- and 180-day survivors was similar to that in the A series. Rat 229, 165-day survivor, which was found dead in its cage one morning, showed nerve regeneration similar to the others but numerous muscle fibres were still small and hypernucleated and there was a fibrous thickening of the adjacent endomysium.

**Rats exposed for 96 hours.**—In all rats the bone, blood vessels and cutaneous tissues were healthy. A 90-day survivor was similar to that in the A series. The 150-day survivor, which was found dead, the 180-day survivor (Fig. 4) and one 365-day survivor, rat 242, all showed numerous small hypernucleated muscle fibres with endomysial fibrous thickening and few or no motor end plates visible. Rat 241, the other 365-day survivor, did not show any fibrous tissue increase and the variation in muscle size was less noticeable. The main and muscle nerves showed a progressive remyelination which in 365 days had almost reached normal.

**Discussion**

The aim of these experiments was to continue the investigation of the histological changes which take place in the tail of the rat, after exposure to conditions comparable with those under which immersion foot develops in man. In previous experiments the tissues had been examined at intervals from 1 to 60 days after exposure. In this further series the process was studied from 90 to 365 days after exposure.

It was found that, under the conditions of the experiment, the main changes, as in the previous series, were in nerve and muscle, the other tissues being relatively unaffected. In nerve and muscle, despite individual variations, there was progress towards the restoration of the histological normal. As might be expected, rats which had been exposed for 48 hours showed a more rapid histological recovery than rats which had been exposed for 96 hours. In both 48- and 96-hour exposures the nerves showed a steadier progress than the muscles. In the 48-hour rats of both A and C groups the nerves reached a state close to normality by 92 days: in the 96-hour rats progress was steady
and similar in the A and C groups, but even after 365 days the average size and density of myelinated fibre in the main nerve was not quite equal to the normal.

In the muscle more individual variation in the extent and degree of persisting abnormality was apparent and it showed itself in the narrow calibre of the fibres and in hypernucleation and endomysial fibrosis. In the 48-hour rats muscle damage was very little except in rats 239 (A/365 day) and 229 (C/165 day). In the 96-hour exposure rats the C series showed more persistent muscle damage and more endomysial fibrosis than the A series. When one considers the extent and severity of the muscle damage seen shortly after exposure in the 96-hour rats it is remarkable what degree of restoration towards normal was shown in 365-day survivors, e.g. rat 243, A series (Fig. 3), and rat 241, C series: on the other hand, rat 242 (C series/365 day) still showed considerable damage and some fibrosis.

The 96-hour rats in which persistently small hypernucleated fibres and endomysial fibrosis were found were those in which few or no motor end plates were visible; whilst motor end plates (even though imperfectly formed and regenerating) were always visible in comparable sections from tails with healthier looking muscles. It is reasonable therefore to suggest that failure to re-innervate and persistent small muscle fibre size run hand in hand, and re-innervation of regenerated muscle fibres is compatible with restoration of normal structure. It was not possible to decide whether the endomysial fibrosis was the cause or the sequel of the failure of innervation. Even in the relatively healthy looking muscle some degree of obstruction of the neurilemma tubes of the regenerating nerves must have been present, for the presence of "escape," "wandering" or "ultraterminal" fibres (Fig. 10) suggests that it was often as easy for the returning stream of axoplasm to burst out of the neurilemma tube as to force its way into the old end plate. Similar appearances are to be seen in animal muscles in which the only pathological process has been nerve crushing or nerve section and suture.

Summary

1. A histological study was made of the changes which occur in the tail of the rat, 90 to 365 days after exposure to cold and wet. The periods and conditions of exposure were comparable with those under which the less severe forms of immersion foot develop in man.
2. As before, the brunt of the injury fell on the neuro-muscular apparatus.
3. The nerves now showed a slow progressive return towards normal.
4. There was a marked individual variation in the amount of persisting muscle damage, which appeared to be correlated with an absence of re-innervation of motor nerve endings.
5. Treatment, in the form of heating up to 37° C. in hot air, appeared to have a slightly adverse effect upon muscle recovery.
Conclusion

From this group of experiments and the previous study it can be seen that the tissues in the rat's tail most directly affected by exposure to cold and wet are the nerves and striped muscles. These tissues degenerate to an extent which depends upon the duration of exposure. The damaged nerves regenerate, axis cylinders first growing down and then becoming myelinated. The damaged muscle fibres also regenerate but fail to regain full histological normality unless they manage to make contact with motor nerves. The extent to which this successful contact is made is in some cases very great and the remarkable degree to which some of these muscles and nerves recover is due, no doubt, to the initial escape from damage of the neurilemma and the sarcolemma, so that there is no serious mechanical obstruction to regeneration.

It is perhaps well to remember that the tail of the rat is very well supplied with arterio-venous anastomoses and may therefore be more able to recover rapidly from spells of cooling than the limbs of man.

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