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THE TOXIC EFFECTS OF TRI-ORTHO-CRESYL PHOSPHATE ON THE NERVOUS SYSTEM

AN EXPERIMENTAL STUDY IN HENS

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While the clinical effects of tri-ortho-cresyl phosphate (T.O.C.P.) on the nervous system of man are now widely known from the papers of Ter Braak (1931), Sampson (1938, 1942), Aring (1942), Staehelin (1942), and of Hunter, Perry, and Evans (1944), the process by which degeneration of the peripheral nerves and the long tracts of the spinal cord is brought about is less well understood. From their original studies Smith and Lillie (1931) concluded that T.O.C.P. was " a specific myelin poison " and that it probably exerted this action by virtue of its solubility in lipoid substances. Aring (1942) from his observations on late cases of T.O.C.P. poisoning in man also believed that this substance had a specific affinity for nervous tissue, but he was unable to suggest a satisfactory mechanism by which nerve and tract degeneration was brought about. One year earlier, however, a new line of approach to the problem was opened up by Bloch's (1941) demonstration that T.O.C.P. inhibited the cholinesterase of horse serum, and it appeared reasonable for him at that time to suggest that the paralysis due to T.O.C.P. might possibly be the result of inhibition of cholinesterase at the myoneural junctions. This view, however, became untenable for it was found that although T.O.C.P. inhibited both serum cholinesterase and tributyrinase, it was ineffective not only against other enzymes such as serum phosphatase and pancreatic lipase (Hottinger and Bloch, 1943) but also against the true cholinesterase of red blood cells and motor end-plates (Mendel and Rudney, 1944). Nevertheless distinction between the two forms of cholinesterase had been thus emphasized. As details of the distribution in the body of serum or pseudocholinesterase came to be worked out, particularly in the nervous system, where it was found to be in greatest amount in the white fibre tracts and in the peripheral nerves (Burgen and Chipman, 1951 ; Ord and Thompson, 1952) it came to be thought possible that it might in some way be concerned with the metabolism of myelin. This was supported by Koelle's (1950) histochemical demonstration that the enzyme was located in the Schwann and supportive glial cells of the nervous system rather than in the conductive elements. Despite the growing amount of information on the localization of this enzyme and its activity *in vitro*, there are as yet no direct indications as to its function *in vivo* or upon what substrates it is active. Largely due to these important gaps in our knowledge there is still much uncertainty as to the part played by inhibition of pseudocholinesterase in the production of the demyelinating lesions in T.O.C.P. intoxication, and this uncertainty is aggravated by striking species differences in the readiness with which changes in the nervous system can be produced by different cholinesterase inhibitors. This lack of correlation between enzyme inhibition and biological effect has been remarked upon by Barnes and Denz (1953) who have cast some doubt upon whether inhibition of pseudocholinesterase by these agents is in fact as important a factor in producing nervous tissue damage as has been hitherto believed.

Turning to the histological aspects of T.O.C.P. intoxication, it is remarkable to find that, although studies by many authors of the changes in both man and experimental animals have yielded the information that demyelination regularly occurs in the peripheral nerves and in certain tracts of the spinal cord, little attention has been paid to the mode of onset of these changes or to determining whether they are primary or merely secondary to degeneration in the axis cylinder. In view of the discrepancies briefly outlined above between the functional disorder and the biochemical changes, a closer and more detailed examination of the pathological process as it affects the nervous system would seem to be warranted, since, for further

progress, an answer must be sought as to whether the demyelination is primary or not. If it is primary, then some additional support will be provided for the hypothesis that pseudocholinesterase resident in the glial and Schwann cells plays some part in the nourishment of the myelin sheath. If, on the other hand, myelin degeneration is secondary to axis cylinder degeneration then it would be more reasonable to look for some biochemical effect of T.O.C.P. upon the neuron itself in order to explain the occurrence of the lesions.

Observations on Paralytic Changes after the Administration of T.O.C.P.

Although the main features of the functional disturbances of the nervous system that follow the administration of T.O.C.P. have been noted by other workers, since this study is particularly concerned with correlating the earliest structural changes with the appearance of functional disorder, a brief note on the present findings would not be out of place. Hens were exclusively used because of the uniform manner with which they react to a single oral dose, and because the effects upon the pseudocholinesterase of both serum and nervous tissue have been closely studied in these animals by Earl and Thompson (1952). Birds of mixed breed were employed aged from 6 months to 2 years, and the T.O.C.P. (Geigy Pharmaceutical Laboratory, Ltd.) was administered in doses of 1 ml./kg. body weight by means of a rubber-tipped glass pipette introduced into the oesophagus.

The birds were examined and exercised daily, and although in the days immediately following poisoning they usually appeared to be in good health, it was observed that in fact there was a steady drop in weight of about 50 g. each day, which figure represented 2 to 3% of their initial weight. The weight loss continued for six to eight days but thereafter with the onset of paralytic symptoms it became less marked and a slight gain in weight was in general noted in the subsequent weeks. It is not clear whether this early loss of weight was the result of anorexia or of some other factor. The crops were always found to be distended with food when opened at necropsy.

The earliest evidence of damage to the nervous system began to appear in a few birds on the eighth day, though more generally on the ninth and tenth days. This was manifested by an unwillingness to walk and when made to do so they tired easily and would prefer to squat. After exercise a decided broadening and clumsiness of the gait could be elicited. When repeatedly made to fly for a short distance, although the first landings were executed normally, subsequently they became more and more clumsy and unsteady during the first few steps after landing. Invariably on the day following these first signs of incipient paresis overt weakness and clumsiness were evident even without previous exercise. The feet would slap heavily on the floor, were clumsy and irregular in their action and were widely spread to maintain balance. During the subsequent four or five days the legs became progressively more weakened until the bird was unable to stand. Examination during this period showed that the ankle jerks were uniformly depressed and the legs were hypotonic. Power was severely reduced in the ankle and knee movements but the upper parts of the limbs were still able to perform quite strong actions though these were poorly coordinated. Changes in sensation were difficult to elicit. In the remainder of the body, although the wings were sometimes noticeably weakened, they were never affected to the same degree as the legs. No impairment of the head and tail righting reflexes was ever observed.

By the end of the first fortnight, therefore, most birds were severely paralysed in the legs, but a few were still able to stand and in these ataxia was conspicuous. Once the condition had reached this stage there was little evidence of progression of the functional damage; the birds, however, frequently did poorly and failed to gain weight. Not infrequently they died unaccountably during the third and fourth week after poisoning. This last event, it was found, could be avoided without materially affecting the extent and severity of the paralysis by reducing the dose of T.O.C.P. to 0·75 or 0·5 ml./kg., and birds on these doses after an initial setback during the onset of the nervous damage gained weight and maintained good general health. With a dose of 0·25 ml./kg. only minimal functional changes in the nervous system could be discerned. Long-term studies of the regenerative capacity of the peripheral nerves after paralysis were not undertaken, but in view of the absence of gross damage to the anterior horn cells of the lumbo-sacral cord a certain amount of recovery of function would be predicted at least in the peripheral nervous system.

Methods

The birds were sacrificed by decapitation at various times from the third day onwards, particular attention being paid to the period immediately before and during the onset of the paralysis. After the skin had been stripped off, each sciatic nerve was exposed in turn from the trochanter of the femur to the ankle joint and portions from both distal and proximal regions were allowed to adhere to perforated card without shrinkage and dropped into 1% osmic acid to fix for 48 hours. Pieces adjacent

to these and from other parts of the nerve were similarly treated but fixed in formol-saline. The brachial nerve was taken in many instances, but chief attention was paid to the sciatic nerve and its branches. The brain and spinal cord were exposed and then allowed to fix for 48 hours in formol-saline before being dissected out together with posterior root ganglia at several levels. Blocks of muscle from foot, leg, and thigh were taken in many instances, but other non-nervous tissues were not systematically examined except with the naked eye.

The peripheral nerves that had been fixed in osmic acid were dissociated in glycerine, washed, and mounted in glycerine jelly. By this method it was judged that minimal distortion of the myelin sheaths had been incurred and at the same time quite considerable lengths of individual fibres, sometimes including four or five consecutive nodes of Ranvier, could be readily examined. Similar dissociated preparations of nerve fixed in formol-saline and stained with sudan black B were also examined for comparison. Frozen sections of both nerves and of spinal cord were stained with sudan black B and with the Smith-Quigley iron-haematoxylin method for myelin sheaths. Paraffin-embedded material from proximal and distal regions of the sciatic nerves and from different regions of the spinal cord and brain-stem, in many cases both in longitudinal and in transverse section, were stained routinely with haematoxylin and eosin and by the method of Glees and Marsland (1952) for axis cylinders. This last method was found to be particularly simple to execute and very constant in its results. The metallic methods of Cajal and of Hortega were employed as the occasion required. For degenerating myelin the Swank-Davenport technique was exclusively employed, the blocks after treatment being embedded in paraffin and sectioned at 10 μ. These were counterstained either with safranin or with haematoxylin and eosin.

Changes in the Peripheral Nerves

Myelin Sheaths.—No undoubted degenerative changes are evident in the myelin sheaths of the peripheral nerves during the first week following the administration of T.O.C.P. The internodal regions of the sheaths are smooth and regular and with polarized light the clefts of Schmidt-Lantermann are everywhere readily visible. Except for some irregularity on either side of the nodes of Ranvier, present in both osmic acid and in formol-fixed material, the significance of which is not clear, there is no hint of the changes that were to be observed during the course of the second week and thereafter.

From the eighth or ninth day there began to appear, first in a few fibres and later in increasing numbers, early signs of the myelin breaking up. This began with the formation of globules of various sizes out of the myelin sheath more or less simultaneously throughout the whole length of the internodes (Fig. 1). At the same time there was loss of the normal anisotropic reaction of the sheath to polarized light. The onset of myelin disintegration, therefore, appeared to resemble very closely the onset of the changes of Wallerian degeneration that follow interruption of the axis cylinder, and the subsequent alterations of the lipoid globules were of the same character. On following individual fibres in dissociated preparations it was apparent that this change was not confined to particular internodal segments, but was continuous along the whole length of the individual fibres studied. All fibres, however, were not affected in this way at the same time and from the twelfth day onwards sheaths in different stages of disintegration were encountered lying beside unaffected fibres. It is difficult, indeed, to state when exactly the onset of degeneration ceased, but by the end of the third week the changes in the affected sheaths were all in a more or less advanced state and there was little evidence of any fresh changes. It is noteworthy that fibres of large diameter were predominantly involved and those of medium size were less conspicuously

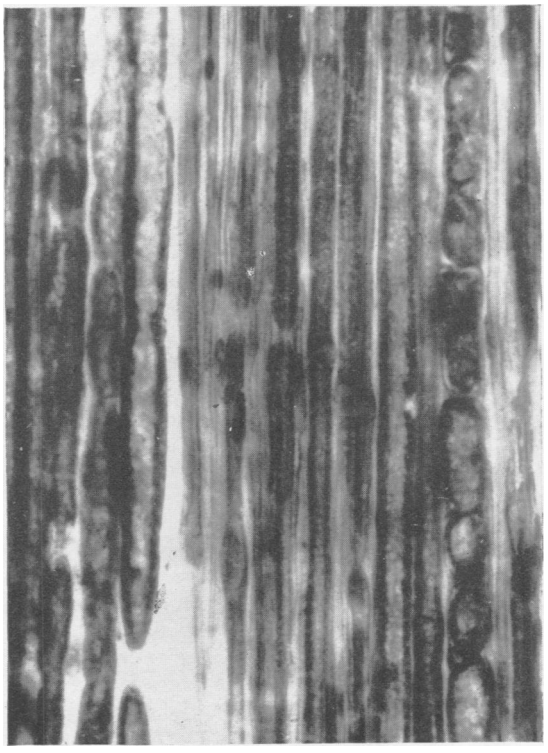

FIG. 1.—Sciatic nerve 14 days after poisoning, showing one sheath in a moderately early stage of degeneration and another just beginning to break up into globules. Frozen section stained with sudan black B. × 830.

affected, with the result that predominantly muscular branches of the sciatic nerve were more severely damaged than those passing to the extremity that contain chiefly cutaneous sensory fibres (Fig. 2). On the other hand, studying a nerve at different levels, such as the anterior tibial, shows that there is a greater amount of degenerating tissue distally than there is proximally, a finding that fits in with the view that these changes are Wallerian in character and are extending peripherally in the expected manner.

Axis Cylinders.—As with the myelin sheath, no definite change can be appreciated in the axis cylinders until the beginning of the second week, when *pari passu* with the myelin sheath changes, individual axons begin to show ballooning, nodularity, and fragmentation. These affected fibres are scanty on the eighth and ninth days but thereafter they become more numerous. Again it is evident that there are a greater number of damaged fibres in the distal parts of the nerves than there are more proximally (Figs. 3 and 4) and different fibres do not show the same stages of degeneration at any one time. With the idea in mind that degeneration might be occurring earlier in the distal parts of the limb than in the proximal, a search was made in the muscles of the lower leg, but in no instance was there found any more severe degree of nerve degeneration than could be accounted for by the amount of change higher up in the nerve, a finding in keeping with the probable secondary nature of the degeneration.

Changes in the Cells of Schwann.—Before the onset of the phase of secondary degeneration, that is during the first week after poisoning, no visible alteration in the appearances of the Schwann cells could be discerned, but following the disintegration of the axon and the myelin sheath the cells of the affected fibre begin to proliferate and become conspicuous after the twelfth to the fourteenth day after poisoning (Fig. 5). At about the same time macrophages were evident in the affected fibres, but foam cells did not make their appearance to any conspicuous degree until about the twentieth day onwards. Droplets of scharlach-staining lipids were not visible until the fourth week after poisoning. It is apparent therefore that the normal process of cell proliferation occurs here but on a reduced scale as it does following nerve section and no evidence is found to suggest that T.O.C.P., at any rate for the time periods studied here, affects cell proliferation in the nerve changes that this substance produces.

Changes in the Spinal Cord and Brain-Stem

The Fibre Tracts.—Examination of Swank-Davenport preparations of the spinal cord from the twelfth day onwards after poisoning shows substantially the same distribution of degenerative changes that have been noted by other authors. The ventral tract of large, heavily myelinated fibres is severely affected in the thoracic and lumbosacral regions (Fig. 9) and is less extensively involved in the cervical regions, where the dorso-lateral tracts (spino-cerebellar tracts) are predominantly attacked. Degeneration in the latter can be traced along the lateral aspects of the medulla into the cerebellum. Degeneration is also present in the upper thoracic and cervical regions in the posterior columns in a narrow band on either side of the dorsal septum. The tract can be traced up to the cervico-medullary junction at which point the fibres end in association with the secondary sensory neurons as in other species. There is never the same degree of change in this last funiculus that is found in either of the other two. Scattered fibres in the other regions of the white matter occasionally, but not constantly, also show evidence of disintegration of myelin with this technique.

In the brain-stem tract degeneration is not so constant as it is in the cord, but not infrequently individual fibres can be picked out in the dorsal cochlear commissure and in the medial longitudinal fasciculus (Fig. 6) while a degenerating fibre is occasionally to be found in the midbrain. It is probably of significance that both the medial longitudinal fasciculus and the fibres of much of the vestibulo-cochlear system are heavily myelinated and that they are tracts of considerable importance in the reflex pathways of the balancing mechanisms that are so highly developed in birds.

Silver-impregnated preparations show that, in all the regions described, where there is myelin fragmentation there is also evidence of axis cylinder disintegration, the fibres being swollen, ballooned, and granular (Figs. 7 and 8). Not all the fibres are affected in any one tract and, as in the peripheral nerves, they do not begin to degenerate at the same time. The fact that the greatest amount of degeneration in the ventral tract occurs caudally and that in the dorso-lateral tracts occurs rostrally indicates that the same peripheral progression of the change is taking place in these tracts as has been noted in the peripheral nerves, and in the same manner it indicates that the changes are essentially Wallerian or secondary in character. The morphological changes in the axon and the fact that disintegration of the latter is in sufficient abundance to account

FIG. 2.—Marchi preparation of the sciatic nerve cut in longitudinal section. The branch to the main muscles of the leg shows many degenerating fibres, while the other, which passes to the lower leg, is scarcely affected in this case. Seventeenth day. Swank-Davenport. × 55.

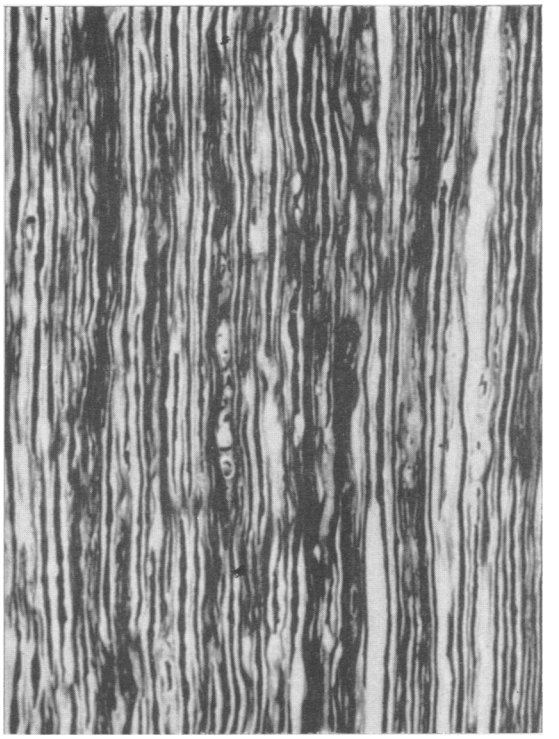

FIG. 3.—Sciatic nerve from the proximal part of the thigh showing only a very low fragmenting fibres. Glees and Marsland silver stain. × 239.

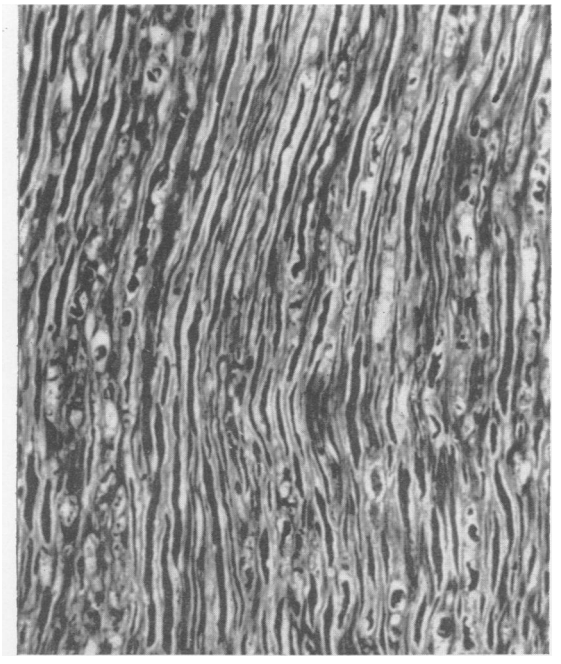

FIG. 4.—Sciatic nerve from the distal part of the same thigh as Fig. 2 showing a relatively large number of degenerating fibres in different stages of disintegration. Glees and Marsland silver stain. × 239.

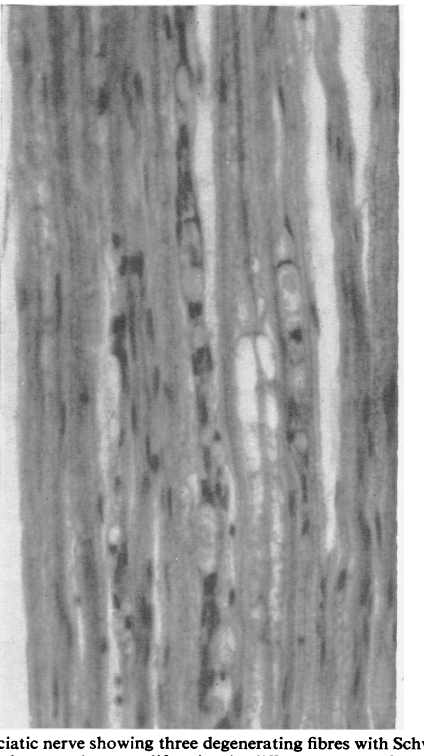

FIG. 5.—Sciatic nerve showing three degenerating fibres with Schwann cell and macrophage proliferation in different stages of progression. Haematoxylin and eosin. × 350.

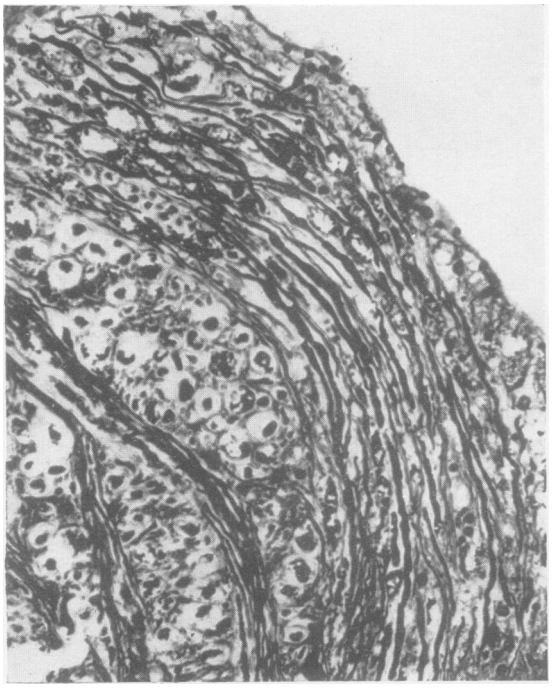

FIG. 6.—Transverse section of medulla 13 days after poisoning, showing fragmentation of a few fibres in the dorsal cochlear commissure. A few ballooned and fragmenting fibres are also visible in the medial longitudinal fasciculus cut transversely. Glees and Marsland silver stain. × 285.

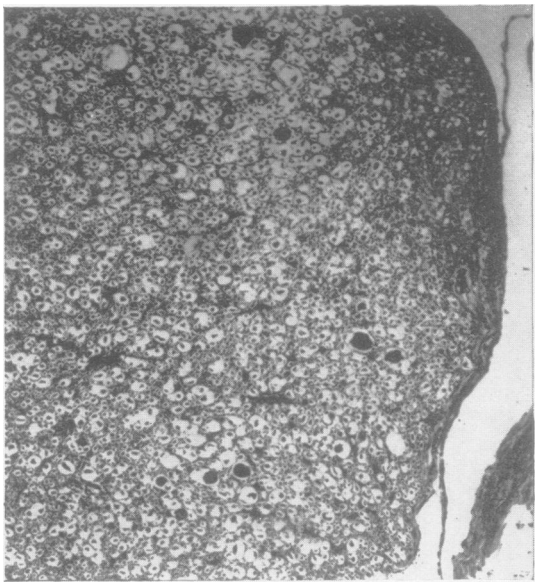

FIG. 7.—Swollen axis cylinders in the spino-cerebellar tract seen in transverse section in the upper cervical region 20 days after poisoning. Same stain as Fig. 5. × 105.

satisfactorily for the changes in the myelin sheath give support to this concept.

Despite a systematic search for an initiating lesion in the different regions of the spinal cord, it was not possible to discern any changes in the nervous tissue before there were definite symptoms of damage; it is apparent, therefore, that these nervous symptoms are the result of the degeneration in the main fibre tracts and are probably not due to any other mechanism.

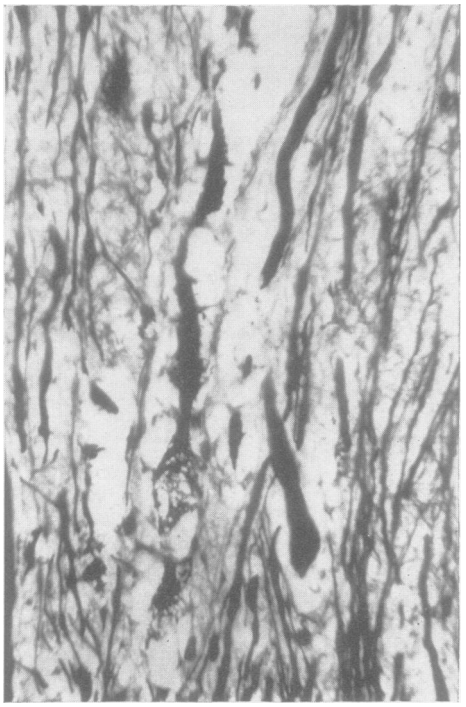

FIG. 8.—Swollen and granular fragmenting axis cylinder seen in longitudinal section in the ventral tract of the thoracic cord 13 days after poisoning. Same stain as Fig. 5. × 465.

The Nerve Cells.—Since it is evident that axonal degeneration occurs both in the central tracts and in the peripheral nerves it might be expected that changes either of a primary or of a secondary nature might be present in the nerve cells of the affected fibres. Examination of the spinal cords up to the thirty-fifth day after dosing with T.O.C.P. shows, however, that there are no changes in the nerve cells that could be held to account for the extensive damage to the nerve fibres, and this is in accordance with the observations of other workers. Except for the presence of one chromatolytic cell in the dorso-medial column in the lumbar region in one case at

the twenty-eighth day, there was also no evidence of secondary chromatolysis, a finding which is surprising in view of the extensive damage that was frequently met with in the sciatic nerve. The possibility that T.O.C.P. in some way interfered with this reaction was excluded by experiment, when it was found that an approximately similar degree of chromatolytic change could be produced by surgical section of the sciatic nerve in the presence of T.O.C.P. intoxication as in control birds.

Preparations by the DaFano method to show the Golgi apparatus did not reveal any deviation from the normal during the first three weeks after poisoning. Not infrequently lipoid granules made visible by the chlorate-osmic acid technique were seen in the cytoplasm of the nerve cells. Similar granules were noted by Smith and Lillie (1931); their significance is not clear. No changes of note were discerned in any other cells of the spinal cord or of the brain-stem or cerebellum.

The Glial Cells of the Fibre Tracts.—No alterations in the appearance or the numbers of the glial cells of the spinal cord or of the brain-stem are discernible during the first two weeks after the administration of T.O.C.P. Thereafter there is a slight numerical increase in microglia, and astroglia also begin to multiply in the degenerating tracts, becoming moderately numerous by the fourth week. During the crucial first eight to 10 days before the onset of severe symptoms no changes can be observed in the oligodendroglial cells.

A Note on Some Features of the Anatomy of the Important Tracts of the Spinal Cord of the Chicken

The anatomical arrangement of the spinal tracts of birds is unlike that of other vertebrates in several important respects. The most significant difference is the absence of a cortico-spinal tract, the cerebral cortex of birds being small and having connexions only with the diencephalic and midbrain regions (Huber and Crosby, 1929). The place of the pyramidal tract of other species appears to be taken by the large-fibred ventral tract that runs the whole length of the cord and can be traced rostrally into the ventral aspect of the median longitudinal fasciculus on either side of the median raphe. Cordotomy studies performed in the upper thoracic region show this tract to be essentially descending and its fibres pass into the anterior horns (Fig. 10). Its origins, however, are less clear and little mention of them can be found in either Ramon Cajal's (1911) or in Ariëns Kappers, Huber, and Crosby's (1936) volumes on the comparative anatomy of the nervous system. Papez (1929), with whom Swank and Prados (1942) agree, states that this tract arises from the optic tectal region which is very highly developed in birds. Unfortunately no direct reference to original work on this subject is given by Papez, and Boyce and Warrington (1899) and Münzer and Wiener (1898), who carried out ablations of the optic lobes in birds, do not describe degenerative changes in the ventral tract following this procedure. Münzer and Wiener do, however, show with the Marchi technique two small tracts in the lateral columns which they consider spring from nuclear masses in the depths of the optic lobes. These latter tracts are relatively insignificant in comparison with the ventral, both in size and in the degree of myelination, and moreover they do not appear to extend the whole length of the cord. Interruption of the ventral tracts leads to ipsilateral spastic paresis with increased reflexes, so that

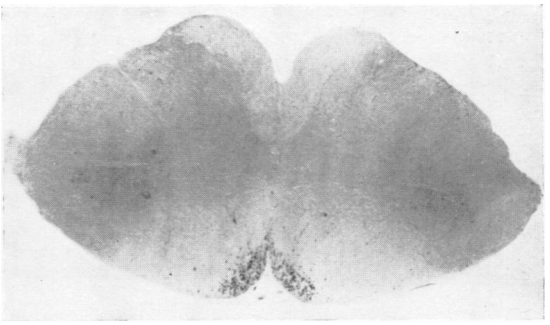

FIG. 9.—Lumbar cord 24 days after poisoning, showing degeneration in the ventral tract. Swank-Davenport. × 18.

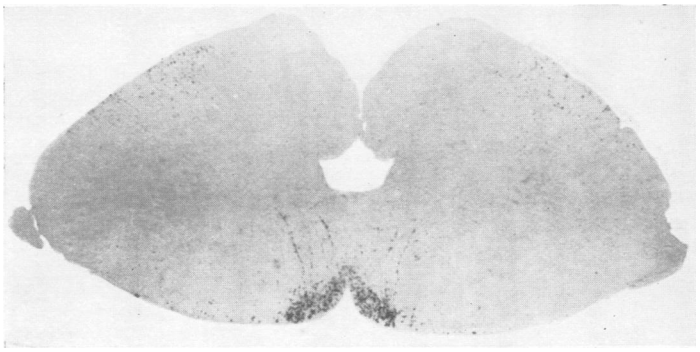

FIG. 10.—Lumbar cord 14 days after upper thoracic cordotomy, showing the degenerating descending fibres in the ventral tract and passing into the anterior horn. The tract is identical in position with that shown in Fig. 9. Swank-Davenport. × 14.

functionally they would seem to resemble the pyramidal tract of other species. Although ablation experiments in the author's hands have not yielded any further information than that already noted above, it is felt that on the available evidence the cells of origin of this tract would be more likely to be found in relation to the midbrain, which is the dominant reflex-controlling centre, than in any other part of the brain-stem. Despite this lack of precise knowledge it is sufficient for the problem in hand to recognize that the fibres of the ventral tract are essentially motor and of much functional importance, and further that the course they run is one of the longest of any in the nervous system of the bird.

The spino-cerebellar tracts of birds, as might be expected from the refined sense of balance of this species, are conspicuous and contain a high proportion of heavily myelinated fibres. The investigations of Friedländer (1898) and of Ingvar (1918) have shown that they arise from the thoraco-lumbar regions and that they contain both crossed and uncrossed fibres. The great majority of these, probably all (Whitlock, 1952), terminate in the cerebellar cortex, distributed chiefly to the anterior folia, while a few scattered fibres pass to the pyramis and uvula of the posterior folia (Whitlock, 1952).

In contrast to the spino-cerebellar tracts the long fibres of the posterior columns of birds are poorly represented and are found in a narrow column on either side of the midline. The great majority of the fibres lying between the posterior horns of grey matter are short inter-calatory fibres linking adjacent levels of the cord and are thus of reflex importance only (Streeter, 1904).

Discussion

Because of its ready susceptibility the domestic hen has been previously used in the study of T.O.C.P. intoxication of the nervous system by Smith and Lillie (1931) and by Sampson (1942), while more recently Barnes and Denz (1953) have shown a similar susceptibility to the other organo-phosphorus compounds di-*iso*-propylfluorophosphonate (D.F.P.) and " mipafox ", both of which produce changes in all essentials identical with those of T.O.C.P. With all three compounds the last authors have shown that by using repeated doses other laboratory animals can be paralysed but with considerably less ease and regularity than can the hen. Man appears to resemble the hen with regard to susceptibility both to T.O.C.P. and to the other organo-phosphorus compounds mentioned, and pathologically the changes are essentially the same in both subjects. It appears, however, that after ingestion of T.O.C.P. there occurs in man a greater amount of recognizable nerve cell damage (Bowden, Turley, and Shoemaker, 1930 ; Goodale and Humphreys, 1931 ; Vonderahe, 1931) than is found in the spinal cord of the hen, chromatolytic change in particular being a feature in the anterior horn cells. Studying the later stages of the condition Aring (1942) found in man degeneration of the pyramidal tracts, the spino-cerebellar tracts, and also of the tract of Goll but not of that of Burdach. In peripheral nerves in all species in which paralysis can be accomplished the sciatic nerves are always more severely and extensively damaged than any other, though nerves to the upper limb may be also affected but to a lesser degree. Not all fibres are attacked in any nerve and mostly only a small proportion show degeneration, but it is evident that the fibres of large diameter and of greatest length are especially chosen, with the result that the distal extremity is more seriously affected and the functional disturbance is predominantly motor rather than sensory. Moreover, a greater degree of degenerative change is visible in the distal part of the nerve than occurs in the proximal part.

In the spinal cord the species differences that are found in the various tracts affected are significant only in that they underline the fact that it is the functionally important tracts containing a high proportion of heavily myelinated fibres of considerable length that suffer damage. It is significant, for instance, that Goll's tract in man is more affected than Burdach's and that the posterior columns are more susceptible in mammals than they are in the bird. These columns in birds have only a fraction of the importance and consequently of the size that they possess in mammals, and it is to be expected that the spino-cerebellar tracts rather than these should bear the brunt of the effects of the poison in birds while in mammals there is a more equal distribution of changes between the two. Of the descending systems, the pyramidal tract in man and other mammals is almost exclusively involved while in birds its homologue the ventral tract is similarly attacked, and it must be apparent that as in the case of the peripheral nerves the functional activity of the fibres is of importance in predisposing them to the toxic action of T.O.C.P.

Since the early investigations of T.O.C.P. intoxication the view has become current that the process in the nervous system is one of primary degeneration of the myelin sheaths, a view based very largely upon the almost exclusive use of the Marchi technique or its modifications. It is clear from the present findings, however, that axis cylinders are destroyed equally with their sheaths and the sequence

of events closely follows that of Wallerian degeneration. Though a careful search was made no evidence could be found for believing that disintegration of myelin was preceding axis cylinder fragmentation by any significant length of time; in dissociated preparations of peripheral nerve it was seen that the myelin changes were similar in all the internodal regions along the length of the individual fibres followed. If the disintegration of myelin was consequent upon some local metabolic inadequacy, which a hypothetical toxic action would imply, then it would be expected that disintegration of the sheath might occur segmentally at least during the early stages of the process, since it is probable that the internodal stretches of sheath are isolated units controlled by the adjacent Schwann cell. Such focal lesions were never observed even in the early stages of the degeneration. Moreover, it is doubtful if myelin degeneration *per se* can produce such a rapid and uniform effect upon its enclosed axon as was observed here, and three dissimilar lines of evidence tend to support this view. The first is the frequent persistence of axons in the plaques of disseminated sclerosis even when they have been stripped of their covering myelin, and the second is the observation of Speidel (1933) that segments of myelin may break down during the development of the amphibian nerve without any apparent adverse effect upon the nerve fibre. Further, Swank (1940) has observed that in starvation in rats the myelin may undergo fragmentation while the axon remains intact.

Attention has been paid in this study to this aspect of the relationship between the myelin sheath and its axon because of the suggestion put forward earlier by Earl and Thompson (1952) that the pseudocholinesterase of the Schwann cells and the glia might play some part in the metabolism of myelin and that the demyelinating action of T.O.C.P. could conceivably therefore be the result of inhibition of this enzyme. As knowledge of the anticholinesterase effects of the organo-phosphorus compounds extended, however, it became apparent that this view could not be sustained (Thompson, 1953), because even though they all appeared to have a more or less equal anticholinesterase effect, only a few were capable of damaging nervous tissue. Moreover, Webster (1954) has shown with the aid of radioactive tracer elements that there is no appreciable change in the turnover of lipoid phosphorus in peripheral nerve during the period of depressed pseudocholinesterase activity following the administration of T.O.C.P. and thus no support is given to the thesis that this enzyme is concerned with the synthetical processes of myelin metabolism. Davison (1953) tested five organo-phosphorus compounds for their anticholinesterase effects and found that although they had an almost equal action upon the enzyme only two, namely D.F.P. and "mipafox", were capable of causing demyelination whereas bis (di-*iso*-propylamino) phosphonous anhydride (iso-O.M.P.A.), O.O. di-*iso*-propyl O. p-nitrophenyl phosphate (iso-E600), and tetra-*iso*-propyl pyrophosphate (T.I.P.P.) had no such effect. Similar results were recorded by Barnes and Denz (1953) and the conclusion was drawn that for the production of demyelination mere depression of the pseudocholinesterase is insufficient. In corroboration it may be mentioned that although demyelination has been produced in ruminants by Smith, Elvove, and Frazier (1930), Smith and Lillie (1931) and Draper, James, and Johnson (1952) only a low activity of pseudocholinesterase can be detected in the brains of sheep and oxen (Gunter, 1946; Mendel and Myers, 1952). On the other hand Mendel and Myers (1952) found in the rat that even though the brain pseudocholinesterase can be reduced to zero activity for several weeks with T.O.C.P. there is no apparent effect upon the health of the animals. On the evidence it is difficult to believe that interference with this enzyme is of primary importance to the nutrition of the myelin sheath in such widely different species as birds and man, while in others it is singularly inert in this respect. The facts strongly suggest that attention should be turned away from the Schwann cells and the glia as being of importance in the pathogenesis of the lesions in T.O.C.P. poisoning and towards the possibility of the neuron being primarily affected.

The main argument in favour of interference with the metabolism of the nerve cell as being the primary change produced by T.O.C.P., and probably also by the other organo-phosphorus compounds that have similar effects upon the nervous system, is the close similarity that exists between the changes produced by these substances and those found in chronic thiamine deficiency. This relationship was initially pointed out by Aring, Bean, Roseman, Rosenbaum, and Spies (1941) and has been stressed more recently by Barnes and Denz (1953). On closer examination these similarities are very striking. The particular susceptibility of the avian species and of man is common to both and in both there is a significant period of delay before the appearance of functional and structural disturbances. Moreover, the pattern of the lesions in each condition is the same, not only in the especial involvement of the distal parts of the fibres, but also in the distribution of the tracts concerned. In the bird the spinocerebellar and the ventral tracts are particularly

picked out in both diseases, while the vestibulocochlear system, as observed here for T.O.C.P. and in thiamine deficiency by Swank and Prados (1942), is also damaged. In neither condition is the perikaryon noticeably affected except in the later stages when cell shrinkage or chromatolysis may be observed. The biochemical mechanism of the changes in thiamine deficiency is now generally accepted as resulting from the slowing of the energy-producing pyruvate oxidation cycle which leads to degeneration of those parts of the nerve cell farthest from the cell body (Swank and Prados, 1942). In T.O.C.P. poisoning Earl, Thompson, and Webster (1953) found that this cycle was unaffected in this condition, but since only brain was studied and not other parts of the nervous system, the possibility that the respiration mechanism is disturbed cannot be completely excluded. The similarities between the two conditions are so close as to suggest very strongly that there may be a common final pathway through which the ultimate changes are brought about.

One further aspect of the problem deserves mention since it has not been fully investigated to date. Both T.O.C.P. and the organo-phosphorus compounds affect aliesterases to a variable degree, though information about this and other aspects of these enzymes is not so precise as it is with the cholinesterase. With T.O.C.P. Earl and his colleagues (1953) found a partial inhibition of tributyrinase to about 50% of its original activity in chicken brain. It would be of interest to know more about this aspect of the question since it is possible to demonstrate distinct differences in the amount of aliesterase in nerve cells. For instance, using the α-naphthol acetate method of Gomori (1952), which presumably visualizes the activity of esterases of the type with which we are concerned, a large amount of enzyme activity can be shown to be present in the anterior horn cells of the spinal cord of the rat and the guinea-pig, while there is practically none in the same cells in the chicken. It would seem reasonable to suggest that even a partial inhibition of enzyme of initially low activity might be sufficient to render it inadequate for efficient cell metabolism. Moreover, variations in the amount of enzyme and its sensitivity might well contribute to the marked differences in the response of the various species to poisoning with these substances. Since the function of the aliesterases in nature is as obscure as that of pseudocholinesterase, the possibility that they may be concerned in the production of the changes in the nervous system due to T.O.C.P. cannot be considered to be completely unworthy of further examination.

Summary

The changes in the nervous system following poisoning with T.O.C.P. have been studied, and degeneration of axis cylinders and myelin sheaths in both the peripheral nerves and long tracts of the spinal cord is found. The degeneration appears to affect the distal extremities of the axons, and long fibres of large diameter are particularly selected.

The closeness with which these changes resemble those of thiamine deficiency is stressed and it is suggested that there may be a mechanism producing neuronal damage common to both conditions.

The relation between damage to nervous tissue and the inhibition of pseudocholinesterase by T.O.C.P. is examined, and it is concluded that this is probably not of primary significance.

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