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CHOLINESTERASE LEVELS IN THE NERVOUS SYSTEM IN TRI-*ORTHO*-CRESYL PHOSPHATE POISONING

BY

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In 1930 Smith and Elvove published a report on a localized outbreak of motor neuritis in the United States. It was shown that the paralysis followed the consumption of a drink known as " ginger jake," and was apparently due to the presence of tri-*ortho*-cresyl phosphate (TOCP) in the fluid extract of ginger used in the preparation of the drink (Smith, Elvove, and Frazier, 1930). Since then cases of paralysis following accidental ingestion of this compound have been described many times (among others, Ter Braak, 1931 ; Germon, 1932 ; Sampson, 1938 ; Aring, 1942 ; Hunter, Perry, and Evans, 1944 ; Hotston, 1946).

The syndrome of TOCP poisoning is usually described as a peripheral neuritis, although it is clear that cord changes are also present. Motor function is severely affected, and the paralysis is more marked in the feet and legs than in the arms. The distal muscles of the limbs are more affected than those nearer the trunk. Sensation seems to be largely unaffected. Complete recovery may ensue, but in some patients late signs may appear of damage to the pyramidal tracts, and the picture then has been described as being very like that seen in amyotrophic lateral sclerosis (Aring, 1942). On histological examination, demyelination is found both in the peripheral nerves and in the white matter of the spinal cord (Smith and Lillie, 1931 : Aring, 1942). Paralysis and demyelination can also be regularly and easily produced by this compound in experimental animals, particularly the hen (Smith, Elvove, and Frazier, 1930 ; Smith, Engel, and Stohlman, 1932 ; Smith and Lillie, 1931).

The study of the immediate cause of demyelinating diseases in man is difficult. It was felt, therefore, that the experimental production of a comparable condition by this compound in animals would be of value in that it would provide an opportunity to gain more insight into the changes in the nervous system that may accompany demyelination. In this instance we should be concerned with demyelination resulting from the action of a single, simple chemical substance, and the condition should therefore lend itself to the study of any biochemical and enzymic changes in the nervous system which might be associated with the histological lesion.

As far as we are aware, the only biochemical effect of trio-*o*-cresyl phosphate which has been described is the inhibition of cholinesterase and tributyrinase activity (Bloch, 1941 ; Hottinger and Bloch, 1943 ; Mendel and Rudney, 1944). Bloch suggested that inhibition of the cholinesterase at the motor end-plates might be the cause of the paralysis. On such a view, however, it would be difficult to account for the

histological changes observed and also the delay of about 14 days before the onset of symptoms.

It is now known that two different cholinesterases are present in the body: the true cholinesterase present at synapses and at the motor end-plates, and the so-called " pseudo-cholinesterase " present in serum and in a number of other tissues, including the nervous system. The true cholinesterase is usually regarded as the enzyme which hydrolyses acetylcholine liberated at nerve-endings, whereas the function of the pseudo-cholinesterase is unknown. Bloch's view, therefore, assumes an inhibition of the true cholinesterase, whereas TOCP appears to be an inhibitor of pseudo-cholinesterase (Mendel and Rudney, 1944; Earl and Thompson, 1952). We have shown that the true cholinesterase present in human muscle and nervous tissue is not significantly inhibited *in vitro* by TOCP. On the other hand, we found a marked and selective inhibition of the pseudo-cholinesterase in human nervous tissue and serum; a similar selective action was also found in the tissues of the hen. We suggested that inhibition of the pseudo-cholinesterase in the nervous system might result in some disturbance of the normal maintenance of the myelin sheaths of nerve fibres, and so cause demyelination. In this connection a recent report (Bidstrup and Hunter, 1952) is of interest in which three cases of poisoning by a new anti-cholinesterase insecticide were described; two of the patients developed a late flaccid paralysis resembling that which follows poisoning by TOCP.

In the work to be described we therefore set out to study the cholinesterase levels in the nervous system of hens poisoned and paralysed by TOCP, in order to determine whether in the poisoned animal as well as under our earlier *in vitro* conditions the same selective inhibition of the pseudo-cholinesterase is found.

Materials and Methods

Hens weighing between 1,400 and 3,000 g. were used in this work. They were poisoned by a single dose of TOCP (1 ml./kg. body wt.), administered by mouth with a 2 ml. graduated pipette.

Blood was taken by venepuncture from the wing vein, and was immediately heparinized. Animals were killed by decapitation at varying intervals (up to 21 days) after intoxication. The whole brain and spinal cord were removed immediately after death, dissected free from membranes, and homogenized in 0.025 M–$NaHCO_3$ to give 1 in 15 (w/v) dilutions.

Cholinesterase determinations were carried out manometrically in the Warburg apparatus at 38°. CO_2 evolutions were measured for periods up to 1 hr. Results for plasma are expressed as μl. CO_2/ml./min. and for tissues as μl. CO_2/g. (wet wt.)/hr.

Tri-*o*-cresyl phosphate, prepared from pure *o*-cresol, was obtained from Geigy Pharmaceuticals, Ltd.

Substrates.—In order to differentiate between the true and pseudo-cholinesterases that may be present in a given tissue preparation we have used " selective " substrates. For the true cholinesterase we have used 0.03 M-acetyl-β-methylcholine chloride (Savory & Moore, Ltd.), which was shown by Mendel, Mundell, and Rudney (1943) to be readily hydrolysed by this enzyme, though attacked only very slowly by the pseudo-cholinesterase of most animal species. In the course of this work, however, we discovered that this substrate is hydrolysed by the pseudo-cholinesterase of chicken plasma, rather more rapidly than by the corresponding enzyme in most other species. In the hen, therefore, acetyl-β-methylcholine is an appropriate substrate for the true cholinesterase when this

enzyme is present in the tissue preparation in much larger amounts than the pseudo-cholinesterase (as in brain), although it is not an ideal selective substrate when much of the latter enzyme is present. This point will be referred to later.

Butyrylcholine, on the other hand, is hydrolysed rapidly by the pseudo-cholinesterase (Stedman, Stedman and Easson, 1932), but only very slowly by the true cholinesterase (Nachmansohn and Rothenberg, 1945). For the estimation of pseudo-cholinesterase activity we have therefore used 0.03 M-butyrylcholine chloride (B.D.H., Ltd.), or butyrylcholine perchlorate, prepared by Dr. G. R. Webster in this laboratory. Butyrylcholine chloride was employed in the earlier experiments, but the perchlorate was substituted in the later ones, since it was found to be a more satisfactory substrate, in that it is much less deliquescent than the chloride. Both salts were found to be hydrolysed at the same rates by human serum cholinesterase.

RESULTS

Estimations of cholinesterase levels have been made on 24 poisoned birds. For about ten days after poisoning the birds remained apparently normal. At any time from the tenth to the fourteenth day the birds showed the first definite signs of paralysis; they appeared quite normal in the cage, but when let out they walked unsteadily. At a later stage, perhaps the next day, the birds would be seen sitting on their heels in the cage; when released they walked unsteadily at first, and after a few minutes they were unable to lift their heels from the ground. In a few days the legs became completely useless and the birds sat with their legs outstretched, unable to move. In some birds the claws were flexed and in others they were extended. Birds in the most severe stages showed some weakness of the wings, and some of the most severely affected showed slow respiration with gaping of the beak. Apart from the paralysis, however, they seemed healthy. They held their heads high and their combs were bright, and except in advanced stages of paralysis they ate well.

A summary of the signs shown by the poisoned birds at the time of killing is given in Table I. Only two birds in the series died, one two days after poisoning,

TABLE I

SIGNS SHOWN BY HENS POISONED WITH TOCP (1 ML./KG. *per os*) AT TIME OF KILLING

(All birds killed before the 8th day appeared normal)

Days after poisoning	Symptoms
8	Slight leg weakness after exercise.
8	Nil.
10	Nil.
12	Nil.
12	Slight leg weakness. Walks on heels.
14	Unable to stand. Walks on heels.
14	Unable to stand.
14	Unable to stand. Sits with legs outstretched.
15	,, ,, ,, ,, ,, ,, ,,
15	Unable to stand.
17	Unable to stand. Sits with legs outstretched.
19	,, ,, ,, ,, ,, ,, ,, Wings weak.
19	,, ,, ,, Wings weak. Gaping.
20	Slight leg weakness. Unsteady gait.
21	Unable to stand. Ataxic.

and the other a few minutes after a venepuncture on the 14th day. The causes of death were undiscovered.

Plasma cholinesterase level.—In view of the sensitivity *in vitro* of the pseudo-cholinesterase of the plasma to inhibition by TOCP, estimations of this enzyme were made on blood samples from 26 poisoned birds, in some serially. Estimations were also carried out on samples withdrawn from all birds immediately before poisoning. By this means it was hoped to obtain evidence of adequate absorption of the orally administered poison. Estimations were also made on plasma samples from a further 20 normal birds. Repeated estimations on the plasma level of a given normal bird showed no great variation. Although the range of the level of plasma cholinesterase (using butyrylcholine as substrate) in different birds is somewhat large (from 10.8–30.0 μl./ml./min.; mean 20.2 μl./ml./min.), there was a sharp fall in the level (Fig. 1), as

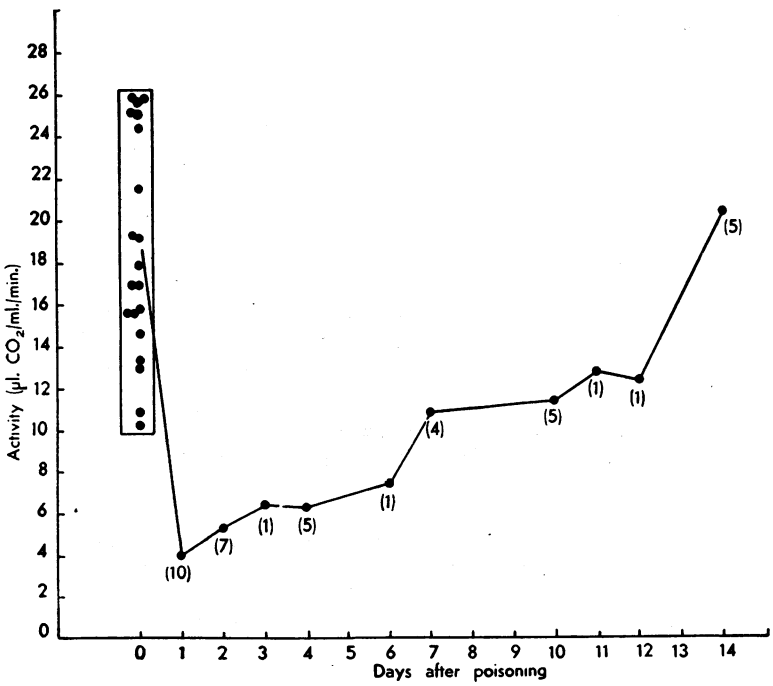

FIG. 1.—Plasma cholinesterase levels in hens after poisoning with TOCP (1 ml./kg. *per os*). (Numbers in brackets denote number of birds used.)

early as 3–4 hr. after administration of the TOCP, to values varying from about 1.6–8.0 μl./ml./min. The plasma cholinesterase activity remained at a low level for 4–6 days, after which it rose slowly, reaching the normal range by about 14 days. It is of interest that TOCP, which is a rather viscous oil, is absorbed sufficiently rapidly from the hen's gastro-intestinal tract to show approximately maximum inhibition of the plasma enzyme by 3–4 hr.

Cholinesterase levels in the brain.—The levels of true and pseudo-cholinesterase were estimated in the brains of 23 animals killed at varying times after poisoning

TABLE II

MEAN CHOLINESTERASE (CHE) VALUES IN NERVOUS SYSTEM OF NORMAL HENS AND OF HENS POISONED WITH TOCP (1 ML./KG. *per os*).

Activity expressed as μl.CO_2/g./hr. (Numbers in brackets denote number of birds studied.)

	Days after poisoning	Brain		Spinal Cord	
		Pseudo ChE	True ChE	Pseudo ChE	True ChE
Normal ..	—	2,490 (13)	12,650 (14)	1,815 (12)	2,450 (11)
Poisoned ..	1	728 (2)	11,220 (2)	455 (2)	1,880 (2)
	2	860 (3)	11,790 (3)	305 (2)	1,485 (2)
	3	846 (1)	11,010 (1)	511 (1)	2,286 (1)
	4	972 (1)	11,720 (1)	207 (1)	1,533 (1)
	5	447 (1)	12,600 (1)	—	—
	6	—	—	285 (1)	1,340 (1)
	8	771 (2)	10,900 (2)	375 (2)	1,260 (2)
	10	690 (1)	10,260 (1)	640 (1)	2,100 (1)
	12	934 (2)	12,320 (2)	556 (2)	1,720 (2)
	14	1,201 (3)	10,940 (3)	775 (3)	1,975 (3)
	15	1,295 (2)	11,660 (2)	765 (2)	2,425 (2)
	17	1,527 (1)	13,830 (1)	981 (1)	2,280 (1)
	19	1,487 (2)	12,170 (2)	505 (2)	1,675 (2)
	20	1,323 (1)	10,300 (1)	1,005 (1)	2,100 (1)
	21	1,770 (1)	14,880 (1)	560 (1)	2,001 (1)

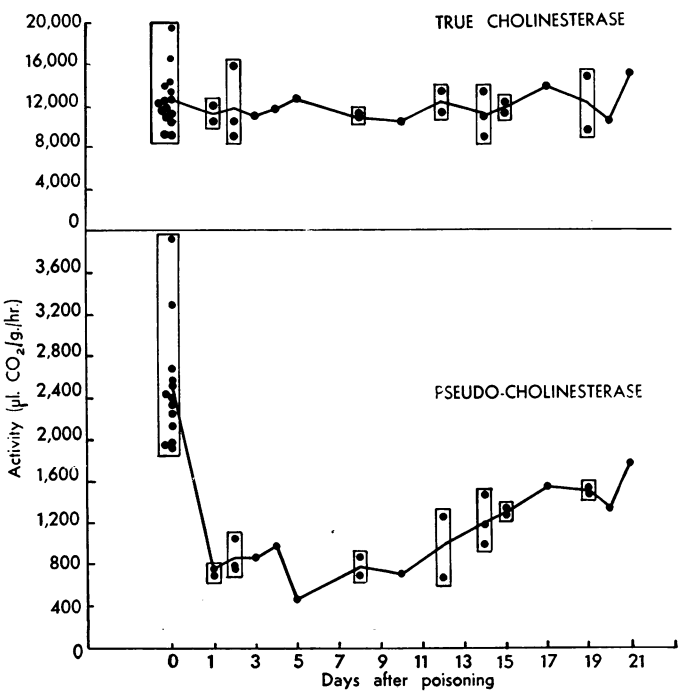

FIG. 2.—Cholinesterase levels in brain of hens after poisoning with TOCP (1 ml./kg. *per os*).

(Table II). In this tissue also the pseudo-cholinesterase activity was markedly reduced one day after intoxication, as compared with the values found in 13 normal birds (Fig. 2); the mean normal level of 2,490 μl./g./hr. (range 1,915–3,930 μl./g./hr.) being reduced to values between 600 and 800 μl./g./hr. It remained at this low value for about 10 days, after which a slow return towards the normal took place. The activity of the true cholinesterase, on the other hand, was unchanged throughout the period of intoxication, all the values falling within the normal range.

Cholinesterase levels in the spinal cord.—Enzyme estimations were carried out on spinal cord preparations from 22 of the poisoned animals killed for estimation of the brain levels. As in the brain, the pseudo-cholinesterase of the spinal cord was found to be profoundly diminished one day after poisoning. The mean normal value of 1,815 μl./g./hr. (range 1,245–2,260 μl./g./hr.) was reduced to about 200–700 μl./g./hr., amounting to a 75–90 per cent inhibition of activity (Fig. 3). This

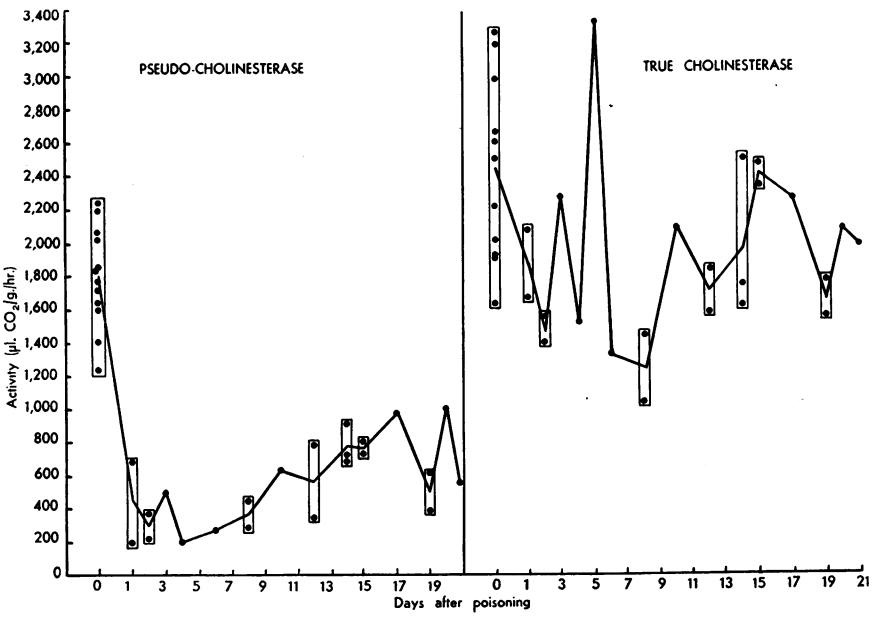

FIG. 3.—Cholinesterase levels in spinal cord of hens after poisoning with TOCP (1 ml./kg. *per os*)

low level was maintained for about 10 days (Table II), as with brain, and although there was a tendency for it to rise later, low values were still found as late as 21 days after poisoning.

The true cholinesterase values in the spinal cord of the poisoned animals showed a slight fall as compared with the normal mean, although most of the estimations fell just within the lower limit of the normal range. This slight fall in the true cholinesterase values in the spinal cord, as compared with the unchanged values in the brain, is probably not due to actual inhibition of this enzyme but to the presence of the relatively large amount of pseudo-cholinesterase in the spinal cord; as we have earlier shown (Earl and Thompson, 1952) the pseudo-cholinesterase in the hen is able to hydrolyse, to a certain extent, the acetyl-β-methylcholine which we were

using as a substrate for measuring true cholinesterase activity. Inhibition of the pseudo-cholinesterase is therefore apparently responsible for the fall in the cholinesterase activity.

Discussion

The main fact that emerges from these experiments is that in hens poisoned with TOCP the pseudo-cholinesterase activity of the plasma and of the brain and spinal cord, removed at intervals after the administration of the poison, is appreciably lowered. The true cholinesterase activity of these tissues, on the other hand, remains almost unaffected. In these respects, therefore, TOCP acting in the intact, living organism behaves as it does under *in vitro* conditions (Earl and Thompson, 1952). The depression of pseudo-cholinesterase activity comes on rapidly, a profound fall being found in the serum three hours after administration of the drug, and in the central nervous system after one day (the shortest time studied). In the brain and spinal cord this fall persists for about ten days at least, after which the activity gradually rises ; in the serum the restoration of pseudo-cholinesterase activity appears to be somewhat more rapid.

It is known that in man the plasma cholinesterase level is lowered in states of malnutrition (Hutchinson, McCance, and Widdowson, 1951). In the poisoned birds, however, loss of weight did not occur until after some days ; the appetite, as already pointed out, failed noticeably only in the advanced stages of paralysis. Since the changes in cholinesterase levels in both the serum and the central nervous system were present and maximal one day after poisoning, it is clear that this was not due to any malnutrition arising from other actions of the poison, or from the paralysis preventing the birds from feeding. From our earlier *in vitro* results, therefore, we are of the opinion that these *in vivo* findings represent a direct toxic effect of the TOCP on the enzyme in these tissues.

We had earlier shown that the true cholinesterase present in human and rabbit skeletal muscle was not affected by concentrations of TOCP that produce an almost complete inhibition of the pseudo-cholinesterase in the central nervous system. Owing to the low level of cholinesterase activity in the skeletal muscle of the hen we have not been able to obtain reliable estimates of the activity of this enzyme in this tissue in poisoned birds. Our finding, however, that the true cholinesterase is unaffected in the brains of poisoned animals, together with the *in vitro* insensitivity to TOCP of this enzyme in skeletal muscle, is not in agreement with the view put forward by Bloch (1941) and revived by Koelle and Gilman (1949) that the paralysis is due to inhibition of this enzyme at the motor end-plates, resulting in an excessive local accumulation of acetylcholine.

From the earlier work in man and animals it seems generally agreed that the primary change in TOCP poisoning is a demyelination. Smith and Lillie (1931) have even described the compound as a " specific myelin poison." Professor G. Payling Wright has kindly undertaken histological studies on material from poisoned birds of the present series and has confirmed the presence of demyelination both in peripheral nerves and in certain of the tracts in the cord ; this work is still in progress and will be described in a later paper.

Tri-*o*-cresyl phosphate is chemically an unreactive compound. Apart from its inhibitory action on pseudo-cholinesterase and its less powerful effect on tributy-

rinase (Hottinger and Bloch, 1943 ; Mendel and Rudney, 1944 ; Earl and Thompson, 1952) it is not known to cause any other biochemical derangement. Experiments are in progress to study its effects on other enzyme systems, and from this work it already seems that the oxidation of glucose and of pyruvate by chicken brain is unaffected by the presence of TOCP *in vitro* and that the brains of poisoned animals oxidize these substrates at the normal rate.

The rapid onset of the fall in pseudo-cholinesterase activity in the poisoned birds, together with the normal behaviour of the birds for about 14 days after poisoning, suggest very strongly that this biochemical effect precedes any structural change in the nervous system, and is not the result of the demyelination. It is possible, indeed, that inhibition of the pseudo-cholinesterase may be responsible for the demyelination.

Many different inhibitors of both pseudo and true cholinesterases, some reversible and others irreversible, are now known. If therefore inhibition of the pseudo-cholinesterase in the nervous system is to be regarded as causing demyelination, it might be expected that some of these other anti-cholinesterase drugs should also cause the same change. We have referred to the two patients reported by Bidstrup and Hunter (1952) who developed a late flaccid paralysis as a result of poisoning by an anti-cholinesterase insecticide, bis-mono*iso*propylaminofluorophosphine oxide, and Barnes and Denz (private communication) have shown that it is possible to produce demyelination and paralysis in chickens both by this compound and by di*iso*propylfluorophosphonate (DFP), the histological and clinical conditions closely resembling those produced by TOCP. Both these compounds inhibit the pseudo and the true cholinesterases and in toxic doses cause initial symptoms of acetylcholine accumulation. In Bidstrup and Hunter's patients these early symptoms were treated successfully with large doses of atropine ; in their animal experiments Barnes and Denz also injected atropine in order to prevent undue activity of accumulated acetylcholine and so allow the hens to survive and show the late development of demyelination and paralysis.

The effects of toxic and even lethal doses of DFP in animals have often been described in the past, and a few cases of poisoning in man by cholinesterase inhibitors have been reported, but the development of a late paralysis of this type has not heretofore been mentioned. This may have been because the poisoned animals died from the effects of the undestroyed acetylcholine in the acute stages of intoxication, before sufficient time had elapsed for these late effects to appear. In survivors, the dose of the poison may have been too small, or its action too short-lived, to cause these late changes.

It seems, therefore, that in addition to TOCP these other two inhibitors of pseudo-cholinesterase can also produce demyelination provided that the animal is tided over the initial cholinergic effects with atropine.

The presence of pseudo-cholinesterase in the nervous system has been demonstrated in a number of different species (Boell, 1945 ; Sawyer, 1946 ; Augustinsson, 1948 ; Zeller, 1949 ; Ord and Thompson, 1950 ; Burgen and Chipman, 1951), but its detailed study has been somewhat neglected in favour of the more abundant true cholinesterase. Ord and Thompson (1952) however, showed that it is present in relatively large amounts in the white matter of human brain (and of the brain in a number of different animal species), and suggested that it might play some part in the

maintenance of the myelin sheath rather than directly with the conducting properties of the axis cylinders. More recently, together with Dr. G. R. Webster, we have shown that human peripheral nerve also contains significant amounts of pseudo-cholinesterase.

There is, unfortunately, little evidence concerning the physiological function of the pseudo-cholinesterase inside the body. Hawkins and Gunter (1946) have claimed that *in vivo* inhibition of this enzyme in the blood and tissues does not elicit symptoms indicative of the accumulation of acetylcholine, and our own observations with TOCP support this conclusion. There is, therefore, no clear reason for assuming that the pseudo-cholinesterase is concerned with the inactivation of acetylcholine. In view of the widespread distribution of this enzyme in the body it is conceivable that it may be generally concerned with some unknown aspect of metabolism, which is of particular importance in the formation or turnover of myelin ; a transient inhibition of this enzymic process might cause no serious upset in the slow but continuous breakdown and formation of myelin, whereas a prolonged inhibition, such as we have shown to be produced by TOCP, might after some time cause defects in myelination to become apparent.

There are, of course, difficulties in the way of accepting the simple view that inhibition of the pseudo-cholinesterase in nervous tissue can result in demyelination. Our findings indicate, however, that the demyelination produced by TOCP is associated with an inhibition of this enzyme in the nervous system, and that this inhibition is an immediate effect which persists for about ten days and therefore precedes the structural and physiological damage.

Summary

1. Hens have been poisoned by the oral administration of a single dose of tri-*ortho*-cresyl phosphate (1 ml./kg. body wt.). Under our conditions this dose regularly causes a paralysis of the legs, which first appears 10–14 days after poisoning. Until this time the birds appear normal.

2. The levels of the plasma cholinesterase and of the "true" and "pseudo" cholinesterase of the brains and spinal cords of hens killed at varying intervals after poisoning have been compared with those of healthy birds.

3. The pseudo-cholinesterase activity of the plasma and of the brain and spinal cord is markedly diminished as early as one day after poisoning, and in the brain and spinal cord remains at a low level for 10 days at least. The true cholinesterase in these tissues is relatively unaffected.

4. It is concluded that these enzymic changes precede the onset of the demyelination caused by this compound. The significance of these findings is discussed in relation to the general problem of demyelination.

We wish to thank Professor G. Payling Wright for the histological examination of nerve tissue, the Chief Superintendent, Experimental Station, Porton, for a grant for the purchase of birds, Dr. G. R. Webster for the preparation of butyrylcholine perchlorate, and Messrs. Geigy Pharmaceuticals, Ltd., for the gift of the tri-*ortho*-cresyl phosphate. Our thanks are also due to Messrs. D. H. Burchett and D. E. Jarvis for their skilled technical assistance.

References

Aring, C. D. (1942). *Brain*, **65**, 34.

Augustinsson, K. B. (1948). *Acta physiol. scand.*, **15**, Suppl. 52.

Bidstrup, P. L., and Hunter, D. (1952). *Lancet*, **262**, 262.

Bloch, H. (1941). *Helv. med. Acta*, **8**, Suppl. **7**, 15.

Boell, E. J. (1945). *J. cell. comp. Physiol.*, **25**, 75.

Burgen, A. S. V., and Chipman, L. M. (1951). *J. Physiol.*, **114**, 296.

Earl, C. J., and Thompson, R. H. S. (1952). *Brit. J. Pharmacol.*, **7**, 555.

Germon, G. (1932). "Intoxication mortelle par l'apiol." Thèse de Paris. Quoted by Hunter, D. (1944). *Industrial Toxicology*. Oxford University Press.

Hawkins, R. D., and Gunter, J. M. (1946). *Biochem. J.*, **40**, 192.

Hotston, R. D. (1946). *Lancet*, **1**, 207.

Hottinger, A., and Bloch, H. (1943). *Helv. chim. Acta*, **26**, 142.

Hunter, D., Perry, K. B. A., and Evans, R. B. (1944). *Brit. J. industr. Med.*, **1**, 227.

Hutchinson, A. O., McCance, R. A., and Widdowson, E. M. (1951). *Spec. Rep. Ser. med. Res. Coun., Lond.*, No. 725, 216.

Koelle, G. B., and Gilman, A. (1949). *J. Pharmacol.*, **95**, 168.

Mendel, B., Mundell, D. B., and Rudney, H. (1943). *Biochem. J.*, **37**, 473.

Mendel, B., and Rudney, H. (1944). *Science*, **100**, 499.

Nachmansohn, D., and Rothenberg, M. A. (1945). *J. biol. Chem.*, **158**, 653.

Ord, M. G., and Thompson, R. H. S. (1950). *Biochem. J.*, **46**, 346.

Ord, M. G.. and Thompson, R. H. S. (1952). *Biochem. J.*, **51**, 245.

Sampson, B. F. (1938). *Bull. Off. Int. Hyg. Publ.*, **30**, 2601.

Sawyer, C. H. (1946). *Amer. J. Physiol.*, **146**, 246.

Smith, M. I., and Elvove, E. (1930). *U.S. Publ. Hlth. Rep.*, **45**, 1703.

Smith, M. I., Elvove, E., and Frazier, W. H. (1930). *U.S. Publ. Hlth. Rep.*, **45**, 2509.

Smith, M. I., Engel, E. W., and Stohlman, E. F. (1932). *Nat. Inst. Hlth. Bull.*, No. 160, U.S. Publ. Hlth. Serv.

Smith, M. I., and Lillie, R. D. (1931). *Arch. Neurol. Psychiat. (Chicago)*, **26**, 976.

Stedman, E., Stedman, E., and Easson, L. H. (1932). *Biochem. J.*, **26**, 2056.

Ter Braak, J. W. G. (1931). *Ned. Tijdschr. Geneesk.*, **75**, 2329.

Zeller, E. A. (1949). *Helv. chim. Acta*, **32**, 484.