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ACUTE TETRAETHYLPYROPHOSPHATE POISONING IN CATS AND ITS MODIFICATION BY ATROPINE OR HYOSCINE

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A number of casualties have occurred with the use of alkyl phosphate anticholinesterases as insecticides, and considerable potential hazard lies in the possible use of such agents in chemical warfare (Wood, 1950). The cause of death in man is not known (Grob, Garlick & Harvey, 1950), although a variety of parasympathetic, neuromuscular and central symptoms occur and respiratory failure is prominent. Experimental animals poisoned with these substances show similar symptoms but, again, the mechanism of death remains imperfectly understood (see review by Gilman & Koelle, 1949).

In the present investigation the acute toxic action of tetraethylpyrophosphate (TEPP) has been studied in the cat, and attention has been directed especially to the factors disrupting respiratory function. Death has been found to be due to paralysis of the respiratory muscles and to cessation of discharge from the respiratory centre, with bronchoconstriction playing a secondary role. The effects of atropine and hyoscine on the course of poisoning have been examined, and the results suggest that these drugs oppose not only the parasympathomimetic but also the central excitant and depressant actions of TEPP. A preliminary account of the present investigation has already been communicated to the Physiological Society (Douglas & Matthews, 1951).

METHODS

Cats were used for all experiments. They were either anaesthetized with a mixture of chloralose 1% and urethane 10% (4–5 ml./kg. intraperitoneally or intravenously after ethyl chloride and ether), or with chloralose (80 mg./kg. intravenously), or pentobarbitone sodium (B.P.) (40 mg./kg. intraperitoneally), or were decerebrated under ether anaesthesia.

Tidal air, intrapleural pressure, respiratory muscle movements, the action potentials in the phrenic nerve and the arterial blood pressure were recorded. To estimate tidal air, records were taken of the air inspired with each breath by having the animal breathe through valves from a spirometer. Intrapleural pressure changes were recorded by introducing a small volume of air into

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the intrapleura space, and following the pressure fluctuations in this by needle cannulae (3 mm. bore) inserted through the chest wall and connected to the pressure recorder described by Wilson (1950).

Movements of the chest and abdominal walls were recorded by following the volume changes in a length of rubber tubing wound round the body wall and connected to a tambour. Diaphragm movements were recorded directly by placing a rubber bellows against the caudal side of the diaphragm, through an incision in the abdominal wall (de Candole, Douglas & Spencer, 1950*a*). The bellows were air-connected to a pressure recorder. In order to display the diaphragm excursions on an oscilloscope this phrenograph arrangement was modified in the following way. The air in the bellows was replaced by 10% saline and the instrument connected to a glass tube ($\frac{1}{2}$ mm. bore) half full of mercury connected to a reservoir of mercury and with electrodes at either end. The resistance between the electrodes depends upon the length of the saline column between them, and this varies continuously with the volume of the bellows and therefore with the contractions of the diaphragm, for mercury is then replaced by saline. This resistance was set up as one arm of a Wheatstone bridge circuit using 1000-cycle a.c. and the out-of-balance voltage recorded. This voltage varies with the resistance, and the balance of the bridge was so adjusted that inspiration gave an increase in voltage.

A sample of the action potentials in the phrenic nerves was recorded by cutting the upper root of one phrenic and placing the central end on platinum electrodes under paraffin. It was not necessary to divide the root longitudinally. The action potentials were amplified and displayed, along with the diaphragm contractions, on a double-beam cathode-ray oscilloscope where they were photographed with a moving paper camera.

Blood pressure was recorded with a mercury manometer.

In order to study the action of TEPP without the complicating factor of anoxia produced by respiratory failure, artificial ventilation was maintained throughout some experiments. Spontaneous respiratory activity in the phrenic nerve and the respiratory muscles still occurred and could be recorded provided the cat was not hyperventilated. In these experiments the vagi were cut and the respiratory movements were found to be independent of the pump rhythm; their strength varied inversely with the ventilation. By opening the chest wall the passive movements of the lungs exert little influence on the records of spontaneous movements of the chest and diaphragm, and similarly these movements do not influence the ventilation of the lungs. An open pneumothorax, with adequate airway, was made by inserting a stout, flanged Perspex tube between two ribs on each side of the chest. The influence of the movements of the lungs on the record of the diaphragm contractions was further kept to a minimum by ventilating at the high rate of about 45/min., thus reducing to a small volume the tidal air required to give adequate gaseous exchange.

Neuromuscular block of the diaphragm was studied in cats with open pneumothorax and under constant artificial ventilation. Both phrenic nerves were dissected out in the neck, and the tied peripheral ends placed on electrodes connected to an electronic stimulator. Contractions of the diaphragm to electrical stimulation of the nerves were recorded by the phrenograph.

In a few experiments the blood pressure was stabilized against the changes normally following the injection of TEPP or atropine. The arterial system was connected through a carotid artery to a reservoir of heparinized cat's blood, kept at body temperature and contained in a rubber bag surrounded by a large volume of air at suitable pressure. This buffer system allowed only very small changes of blood pressure (<8 mm. Hg) during vasodilatation or vasoconstriction, as blood left or entered the reservoir to compensate for the volume change of the vascular system.

RESULTS

An intravenous injection of 0·3 mg./kg. of TEPP caused death within 5 min. in all cats; 0·25 mg./kg. was not always fatal. Respiratory movements generally ceased about a minute after injection, or less with the higher doses, and always failed some time before the heart. There were widespread muscular

fasciculations and frequently convulsions. Death could be prevented by artificial ventilation, although this had no detectable effect on the convulsions and fasciculations; when the artificial ventilation had been maintained for about 40 min. spontaneous breathing started again.

A typical experiment with 0·3 mg./kg. TEPP is shown in Fig. 1. After a brief period of respiratory stimulation, air intake falls away rapidly with diminution of the contractions of the respiratory muscles and of the fluctuations of the intrapleural pressure. No evidence is seen of bronchoconstriction before

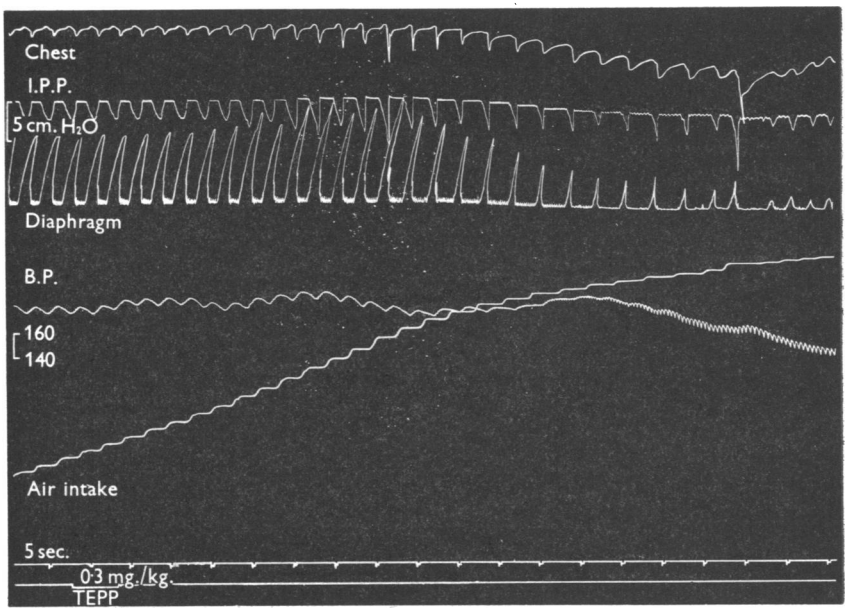

Fig. 1. Cat, chloralose-urethane. Record of chest movements (inspiration downwards), intrapleural pressure, diaphragm movements (inspiration upwards), systemic blood pressure and air intake. At signal, 0·3 mg./kg. TEPP injected intravenously.

respiratory movements fail. Once they have failed any bronchoconstriction present would not be shown in our records. We are therefore unable to state whether it occurred or not at the later stages of the experiment. In any case it cannot have been more than a secondary factor in the development of respiratory failure, which results primarily from abolition of breathing movements.

In only one cat was the effect of TEPP different. Lung ventilation ceased despite persistent muscular contractions and powerful intrapleural pressure fluctuations (Fig. 2). Here the failure is not a weakening of the lung-inflating mechanisms, but a lessening of the distensibility of the lungs. Such an effect arising from bronchoconstriction is a well-recognized feature of anticholin-

esterase poisoning, and this experiment illustrates the profound obstruction to breathing it can cause.

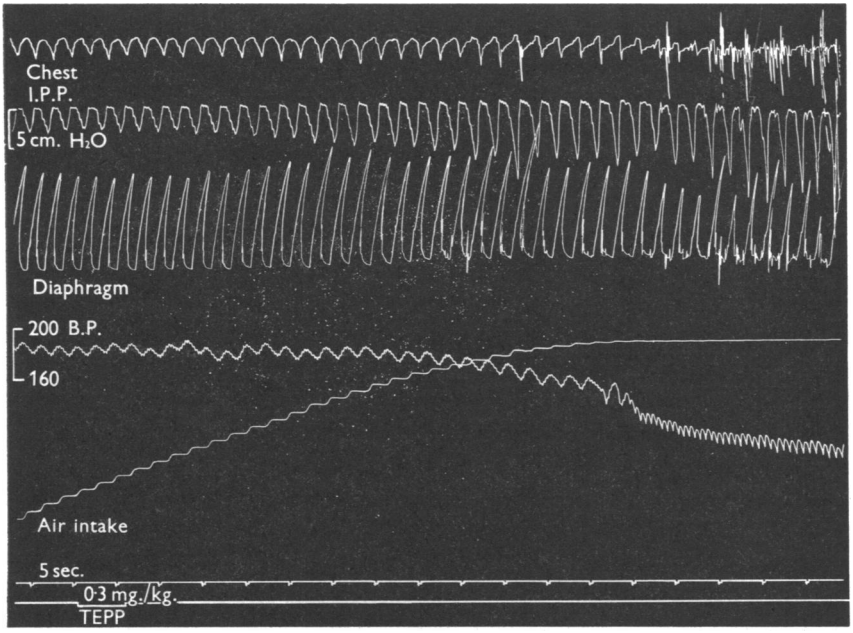

Fig. 2. Cat, chloralose-urethane. Record of chest movements (inspiration downwards), intrapleural pressure, diaphragm movements (inspiration upwards), systemic blood pressure and air intake. At signal, 0·3 mg./kg. TEPP injected intravenously.

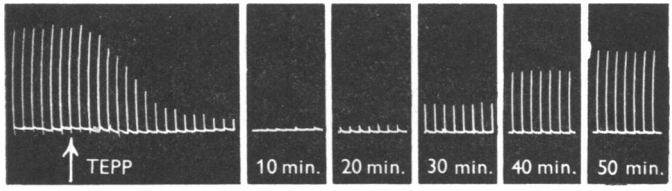

Fig. 3. Cat, chloralose-urethane. Diaphragm contractions to bilateral phrenic nerve stimulation ($\frac{1}{2}$ sec. bursts of maximal shocks of frequency 30 per sec. every 10 sec.). At arrow 0·45 mg./kg. TEPP injected intravenously. Subsequent traces are samples from the continuous record taken 10, 20, 30, 40 and 50 min. later.

Neuromuscular block. If the phrenic nerves are stimulated and the diaphragm contractions recorded, intravenous injection of TEPP (0·45 mg./kg.) may be seen to cause a prompt dwindling of the diaphragm response to repetitive stimulation (Fig. 3). Within a minute or so of injection scarcely any response remains, although direct stimulation of the diaphragm evokes a powerful contraction and, as shown later, the phrenic nerve still conducts. The block is

reversible, and passes off with time. The profound neuromuscular paralysis shown in the experiment of Fig. 3, in which the phrenic nerve is stimulated electrically, persists for more than 20 min. This is longer than paralysis lasts when the diaphragm is contracting to natural spontaneous phrenic discharge. The more prolonged paralysis is probably due to the greater intensity of nervous activity caused by the electrical stimulation which involved stimulation for half a second every 10 sec. with supramaximal shocks at a frequency of 30 per sec. A number of findings indicate such a relationship between intensity of stimulation and degree of block. Diaphragm contractions to single maximal motor nerve shocks, for example, are not depressed but potentiated by intravenous injections of 0·6 mg./kg. TEPP. To show depression to single shocks, either larger doses of TEPP are required, or a short tetanic stimulation has to be interpolated in the train of single shocks (Fig. 4). Further, if stimulation is withheld for a brief period during recovery of neuromuscular function, the first contraction after the pause is larger than its fellows (Fig. 5). And again, during recovery, successive tetanic responses may tend to diminish, yet maintain their amplitude when elicited less frequently (Fig. 6).

Some neuromuscular block also develops in the absence of nerve stimulation. In some experiments the phrenic nerves have been tied, cut and mounted on electrodes. Then control responses have been elicited and TEPP injected in the absence of stimulation. Tests made some minutes later in these experiments showed that considerable block had occurred, although this was obviously less than when electrical stimulation was continuously maintained.

Failure of central respiratory activity. Records of the action potentials in the phrenic nerve show that the respiratory centre fails shortly after injection of TEPP. This failure might be thought to arise from asphyxia consequent on the peripheral actions of TEPP such as neuromuscular block or bronchoconstriction. However, the complicating factor of asphyxia has been countered in a number of experiments by artificially ventilating the cat throughout, the chest being opened, the vagi cut, and the respiratory pump adjusted so that the animal made spontaneous respiratory movements. In these conditions TEPP still caused the centre to fail (Fig. 7). The likelihood that this failure is due to asphyxia is further diminished by the finding that for about 40 min. after TEPP poisoning, central respiratory activity could not be restored by changing the rate of artificial ventilation. In whatever way the minute volume of artificial respiration was varied in individual experiments, the respiratory centre remained quiescent, and death occurred when the pump was switched off. It was only when artificial ventilation had been maintained for about 40 min. that spontaneous respiration reappeared.

The action of atropine and hyoscine on central respiratory failure. Prompt recovery of the respiratory centre could be induced at any time by atropine or hyoscine. Usually within 10–30 sec. of intravenous injection of one of these

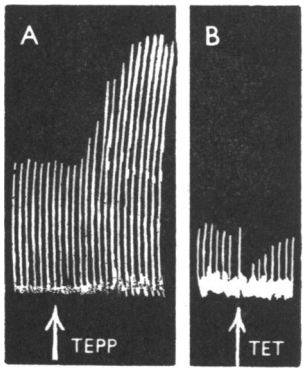

Fig. 4. Cat, chloralose-urethane. Diaphragm contractions to stimulation of both phrenic nerves every 8 sec. with single maximal shocks A. Control.; at arrow 0·6 mg./kg. TEPP injected intravenously. B. After further TEPP injected intravenously (total 1·2 mg./kg.); at arrow a single burst of repetitive stimulation (½ sec. at 30 per sec.) applied to nerve.

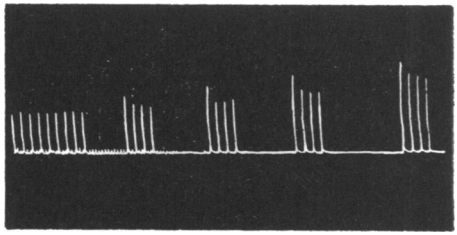

Fig. 5. Cat, chloralose-urethane. Diaphragm contractions. Both phrenic nerves stimulated for ½ sec. every 10 sec. with maximal shocks at 30 per sec. Record taken during recovery after 0·4 mg./kg. TEPP intravenously. Stimulation withheld during periods indicated by gaps.

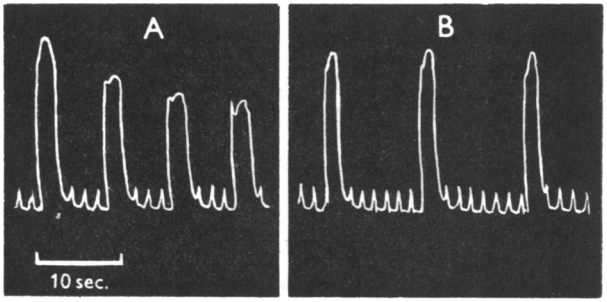

Fig. 6. Cat, chloralose-urethane. Contractions of the diaphragm to repetitive (25 per sec.) stimulation of both phrenic nerves with maximal nerve shocks. In B the stimulus is applied less frequently than in A.

drugs (1 mg./kg.) bursts of action potentials reappeared in the phrenic nerve (Fig. 7), and thereafter the respiratory centre continued to discharge apparently normally for the duration of the experiment; even large doses of TEPP (4–10 mg./kg.) then failed to suppress its activity. But neither atropine nor hyoscine appears to influence the neuromuscular impairment, and how well the

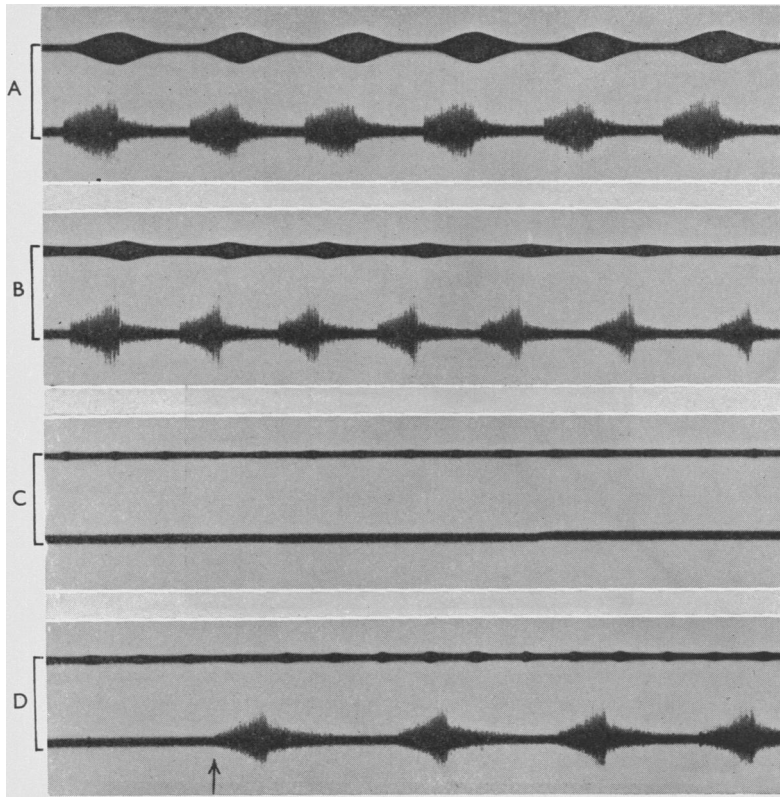

Fig. 7. Cat, pentobarbitone sodium. Both vagi cut. Bilateral open pneumothorax. Constant artificial respiration. Records of spontaneous respiratory activity. Upper record shows diaphragm contractions (indicated by widening of the trace). Lower record shows action potentials in the left phrenic nerve. A. Control. B. 30 sec. after TEPP 0·35 mg./kg. intravenously. C. 3 min. after TEPP 0·35 mg./kg. intravenously. D. 1 min. later, during which time atropine 1 mg./kg. has been injected (arrow indicates the onset of phrenic discharge).

respiratory impulses restore breathing movements depends on the intensity of block present. During the first few minutes of TEPP poisoning when block is profound, little or no muscular movement occurs (Fig. 7), but with time the movements increase, and in about 10–15 min. the block has so lessened that they are sufficiently strong to maintain life (viz. Figs. 9 and 10). Recovery of neuromuscular transmission in the spontaneously breathing animal is obviously

much more rapid than in an animal whose phrenic nerves are stimulated electrically as shown in Fig. 3. An estimate of the course of paralysis to spontaneous phrenic nerve activity is obtained by recording the diaphragm contractions in a cat whose respiratory centre is protected against the actions of TEPP by atropine, and kept in a steady state of activity by artificial ventilation suitably regulated as described for the experiment of Fig. 7. An experiment of this nature is illustrated in Fig. 8. However, the degree of neuromuscular block which occurs in such circumstances is probably somewhat greater than that which exists in experiments where failure of the respiratory centre also occurs, i.e. in cats not given atropine or hyoscine. In the experiments of Figs. 9 and 10, for example, restoration of respiratory centre discharge with hyoscine evoked diaphragm contractions which were powerful in comparison with those

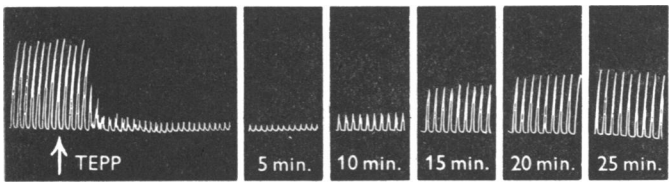

Fig. 8. Cat, chloralose-urethane. Atropine 2 mg./kg. Bilateral open pneumothorax and constant artificial ventilation. Phrenograph record of spontaneous movements of the diaphragm. At arrow, TEPP 0·45 mg./kg. intravenously. Subsequent records 5, 10, 15, 20, 25 min. later.

occurring the same time after poisoning in the experiment of Fig. 8. It seems that the greater spontaneous phrenic nerve activity has resulted in the more profound block, just as was found to be the case for electrical stimulation. Nevertheless, the experiment of Fig. 8 clearly illustrates both the severity of the neuromuscular blocking action of TEPP and its transience. In so doing, it explains fully why atropine fails to prevent respiratory failure yet restores powerful breathing when given some minutes after poisoning.

In combination with artificial respiration, however, atropine or hyoscine affords a considerable measure of protection against TEPP. Each of several cats given atropine or hyoscine (2 mg./kg.) plus artificial respiration has survived intravenous injection of 10 mg./kg. TEPP. Spontaneous breathing reappeared about 40–60 min. after this huge dose.

The action of atropine and hyoscine on convulsions. Although the most striking effect of the atropine alkaloids was to restore central respiratory activity, another action was frequently observed. Thus, when convulsions were present, they were much diminished by atropine or hyoscine. The effect was only observed with the higher doses (1 mg./kg. or more); smaller doses, although controlling parasympathetic effects of TEPP, had little apparent influence on convulsions.

Paralysis of chest muscles. The neuromuscular disturbance of the chest muscles has not been studied in the same detail as that of the diaphragm, but seems to follow a similar pattern. Thus, although atropine maintains the activity of the respiratory centre, the movements of the chest wall dwindle during the height of poisoning as do those of the diaphragm. The chest, however, appears to be less severely affected. This may be seen during the onset of symptoms (Fig. 1), and a similar relationship has been observed during recovery. In one cat, for example, when atropine was given 5 min. after TEPP, movements of the chest reappeared, although the diaphragm was so enfeebled that it rose passively into the chest with each breath and prevented adequate lung ventilation. Some 4 min. later, after further artificial ventilation, the diaphragm also began to contract and spontaneous breathing was then able to maintain life.

Analysis of the action of atropine and hyoscine. Atropine or hyoscine rapidly overcomes the parasympathomimetic effects of TEPP, such as failure of the

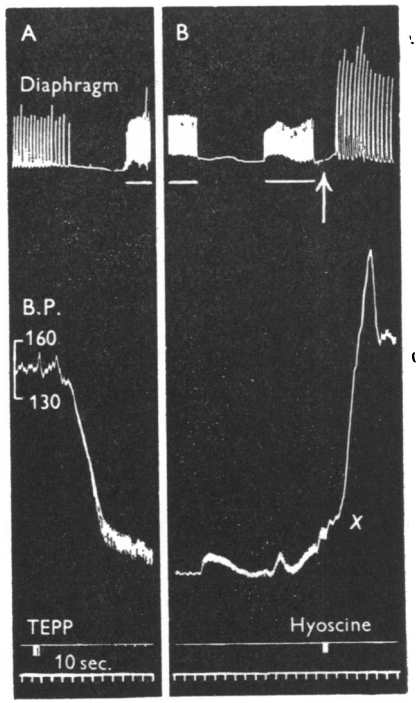

Fig. 9. Cat, chloralose-urethane. Chest intact. Normal respiration. Diaphragm movements (phrenograph) and systemic blood pressure. Artificial respiration given during periods where diaphragm trace is underlined. A. 0·45 mg./kg. TEPP intravenously at signal. B. 11 min. later, showing absence of spontaneous respiratory movements on first test and their reappearance on second test, during which hyoscine (1 mg./kg.) was injected intravenously (at point indicated by arrow). First breath taken at point marked *X* on blood-pressure record.

circulation or bronchoconstriction, and when given during artificial ventilation increases the respiratory exchange. The question therefore arose as to how much these peripheral effects contributed to the restoration of the respiratory centre. This problem was approached by making use of the fact that the neuromuscular block caused by TEPP is more transient than the central respiratory failure, so that after the first few minutes of poisoning the presence or absence of respiratory muscle movements is a reliable index of whether or not the respiratory centre is functioning. It was found that atropine or hyoscine

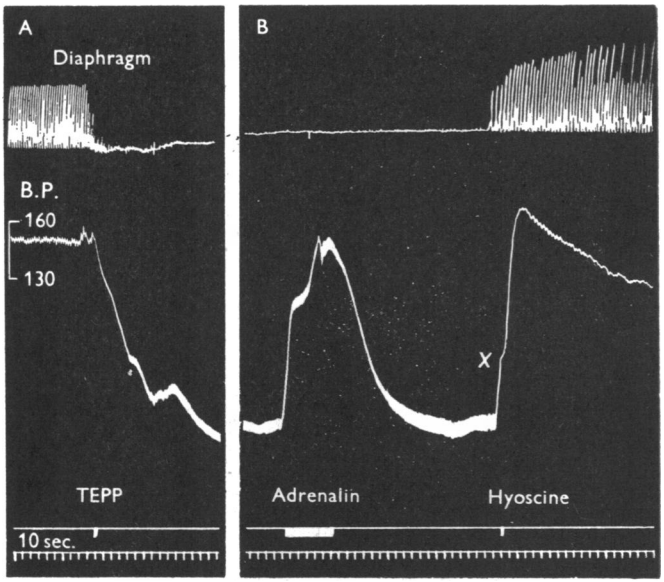

Fig. 10. Cat, chloralose-urethane. Both vagi cut. Bilateral open pneumothorax, constant artificial ventilation. Records of spontaneous diaphragm movements (phrenograph) and systemic blood pressure. A. 0·45 mg./kg. TEPP intravenously at signal. B. 8 min. later; intravenous infusion of adrenaline (150 μg. in 15 ml. saline) and injection of hyoscine (1 mg./kg.). First breath taken at point marked *X* on blood-pressure record.

each restored the activity of the respiratory centre, even when the possibility of its improving lung ventilation was excluded. In experiments such as illustrated in Fig. 9, for example, either drug restored breathing movements when injected during a period in which respiration was withheld. The restoring effect of atropine and hyoscine on respiration was thus not dependent on respiratory changes in the lungs. Nor did it depend on the circulatory effects. Breathing often began before the circulation had been fully restored (at points *X* in Figs. 9 and 10); further, quickening the heart and raising the systemic blood pressure by infusing adrenaline to mimic the effect of the belladonna alkaloids, failed to arouse breathing (Fig. 10); and finally, in experiments in which significant

fluctuations in blood pressure were avoided by using a stabilizing device, atropine still restored breathing movements.

Pentamethonium. Atropine or hyoscine do not overcome the neuromuscular block caused by TEPP. This action still causes death unless artificial ventilation is maintained until it has passed. No satisfactory antagonist to the neuromuscular blocking action of anticholinesterase drugs has yet been described. The block, however, is probably caused by excess of acetylcholine, and a similar block produced by the acetylcholine-like substance, decamethonium, is reduced by pentamethonium (Paton & Zaimis, 1949). It was found that pentamethonium also opposed the neuromuscular paralysis produced by TEPP. In atropinized cats, artificially ventilated, several times the normal paralysing dose of TEPP could be given after pentamethonium without the development of the usual profound block (compare Fig. 11 with Fig. 7).

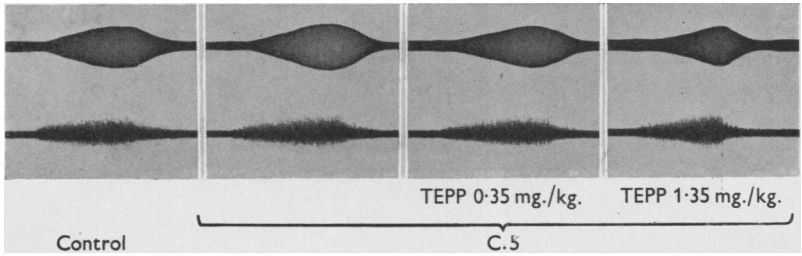

Fig. 11. Legend as in Fig. 7 (same animal 2 hr. later). Atropine 4 mg./kg. Upper record shows diaphragm excursion. Lower record shows action potentials in left phrenic nerve. Representative tracings taken before and after pentamethonium (C5, 15 mg./kg.), showing the effect of two doses of TEPP, 5 and 8 min. after C5.

Trial of the prophylactic effect of pentamethonium in a few atropinized cats breathing spontaneously has shown that while this drug (10–15 mg./kg.) does prevent the precipitate neuromuscular paralysant action of TEPP, it does not prevent death, but only delays it. Experiments in which the phrenic nerves have been electrically stimulated, as in Fig. 3, have shown that the protective effect of pentamethonium at the neuromuscular junction is only partial, and that TEPP still causes neuromuscular block after pentamethonium. This may account for the failure of pentamethonium to prevent death. On the other hand, the failure may arise from some other action of pentamethonium such as block of autonomic ganglia or central depression.

DISCUSSION

The cat is generally considered to be especially susceptible to the parasympathomimetic effects of anticholinesterase poisoning such as bronchoconstriction (Gilman & Koelle, 1949). Our experiments, however, show that doses of TEPP causing rapid death act primarily by abolishing the normal lung inflation forces.

In such circumstances bronchoconstriction seldom plays a significant role. Nevertheless, it has clearly manifested itself in one exceptional experiment where respiratory muscle contractions persisted, and is probably present in most of the TEPP poisoned cats. TEPP (0·2–0·4 mg./kg.) given to cats with open chest under constant negative pressure ventilation (Douglas, unpublished observations) has usually caused a pronounced reduction in tidal air. In different circumstances greater significance might be attached to bronchoconstriction; for example, were the failure of respiratory movements avoided by some treatment; or again, were administration of TEPP by inhalation to favour effects in the lungs. But the finding that bronchoconstriction is of secondary importance in acute death following intravenous injection, and in any event is readily controlled by atropine, focuses attention on the extrapulmonary factors, neuromuscular block and failure of the respiratory centre.

Neuromuscular block. The neuromuscular block which was found to appear within seconds of injection and to cause profound paralysis of the respiratory muscles must be attributed to inactivation of cholinesterase at the end-plate region. Chennells, Floyd & Wright (1949) have shown that the effects of TEPP on the mechanical and electrical responses of the cat's femoral nerve–quadriceps preparation closely resemble those of eserine. Similarly, the actions of TEPP on the diaphragm, namely, potentiation of the single maximal motor nerve shock, depression of the response to repetitive nerve stimulation, and post-tetanic depression of single twitches, are all well-recognized features of cholinesterase inhibition and explained by persistence of acetylcholine. The obvious source of this acetylcholine is the gross activity of motor nerves, either as a result of normal efferent discharge or electrical stimulation of the nerve trunk. But other sources are possible: e.g. random discharge in the nerve cut and mounted on electrodes (Bacq & Brown, 1937); leakage from the terminal fibres of the resting nerve (Feldberg, 1945*a*; Fatt & Katz, 1950); or even carriage in the blood from other sites in the cholinesterase depleted animal. This last possibility has in fact been examined recently by Douglas & Paton (unpublished observations), and considerable quantities of acetylcholine have been found in the circulation of cats poisoned with large doses of TEPP. It is therefore understandable that some neuromuscular block should develop, even in the absence of nerve stimulation, and yet be entirely a consequence of the cholinesterase inhibiting property of TEPP. There is no need to postulate some direct nicotine-like action of TEPP as do Heymans and his school (see Verbeke, 1949).

Throughout, the relation between intensity of nervous activity and the degree of neuromuscular block has been striking; the greater the nervous activity the more profound the block. Such a relationship is the obvious consequence of the action of TEPP being indirect and dependent upon endogenous acetylcholine. It has revealed itself not only when the phrenic nerves were stimulated electrically but also when they were activated normally by the respiratory

centre. Diaphragm paralysis, for example, was comparatively severe in those animals in which the respiratory centre was maintained active by atropine. Indeed, it appears probable that in TEPP poisoning, failure of the respiratory centre to some extent spares the myoneural junction. Consideration of this effect might be of value in treatment. Thus, recovery of neuromuscular function of respiratory muscles might be promoted by suppression of the activity of the respiratory neurones by suitable artificial ventilation, while suppression of convulsions by atropine or barbiturates might be expected to lessen the extent of paralysis of other skeletal muscle.

In the case of TEPP the neuromuscular block following intravenous injection is short-lived, and adequate function reappears within 15 min. when life is maintained by artificial ventilation. Other anticholinesterases such as DFP have a much less transient effect, and attempts have been made to overcome this aspect of poisoning by pharmacological means. Curare and Mg^{++} have both been tried but with little success (see Gilman & Koelle, 1949). Our experience with pentamethonium shows that it has some protective action against the neuromuscular effects of TEPP, but not to an extent to be of importance therapeutically. Nevertheless, the demonstration of such activity in this compound is encouraging and suggests that further trial might be worth while.

Failure of the respiratory centre. The failure of the respiratory centre which followed injection of TEPP was of longer duration than the neuromuscular block. It was the most striking of the effects and obviously a factor of prime importance in the acute toxic action of that substance. Some workers attribute such central respiratory failure to asphyxia consequent upon peripheral effects of the anticholinesterase drug, such as paralysis of the respiratory muscles, bronchoconstriction and lowered blood pressure. Lundholm (1949) offers this explanation for the effect of DFP in the rabbit, and there is some evidence that in the same species TEPP poisoning also involves such a peripheral mechanism (de Candole, Douglas & Spencer, 1950*b*). But the results of our experiments, in which lung ventilation and blood pressure have been controlled, suggest that the central respiratory failure produced in cats by the intravenous injection of TEPP is not brought about in this indirect way. Nerve section shows, further, that afferents in the vagus, aortic or sinus nerves are not involved. A central action of TEPP seems more likely. In this case the restorative action of atropine or hyoscine is of special interest. Here again the control experiments indicate that the effect of these drugs too is independent of their peripheral action in overcoming parasympathomimetic effects. Indeed, the weight of evidence points to the failure of the respiratory centre after TEPP and its resumption of activity after atropine or hyoscine being due to actions of these substances within the central nervous system. A similar antagonism between anti-cholinesterases and atropine on the respiratory centre has been described by

Schweitzer & Wright (1938) using neostigmine, and by Gesell & Hansen (1943) using eserine.

The significance of our evidence, and any bearing it has on the hypothesis that acetylcholine is normally involved in respiratory centre function (see Gesell & Hansen, 1943; Miller, 1949) depends on the answer to a question that is continually raised by such studies, namely, whether the effects of these drugs are entirely referable to their influence on the actions of endogenous acetylcholine. Certain facts favour the view that TEPP acts entirely by allowing accumulation of acetylcholine. Inhibition of cholinesterase is the only action of this drug which has yet been demonstrated. Moreover, acetylcholine has been found to have central effects on respiration, depression as well as excitation (see review by Feldberg, 1945*b*). And finally, these central actions of acetylcholine on the respiratory centre are opposed by atropine (Gesell & Hansen, 1943).

On the other hand, it would be dangerous to conclude that all central actions of an antagonism of acetylcholine are explained by its annulling the effects of acetylcholine. For instance, both atropine and hyoscine are antagonists to acetylcholine as well as to the anticholinesterases, but the main central actions of atropine are excitant, those of hyoscine depressant. It is impossible to say at the present stage of our knowledge to what extent these effects are the result of an antagonism of central actions of acetylcholine.

Whatever the mechanism involved in the action of these drugs on central respiratory function, the fact remains that atropine or hyoscine protects against, or abolishes, the effect of TEPP on central respiratory mechanisms. This action, together with control of parasympathomimetic effects, allows the cat to survive huge doses of TEPP provided the transient neuromuscular block is countered by artificial ventilation.

Convulsions and the action of atropine and hyoscine. The convulsions produced by TEPP are, by their co-ordinate nature, obviously of central origin. The fact that they are suppressed by atropine or hyoscine is thus further evidence of a central antagonism between these belladonna alkaloids and the anticholinesterases. Antagonism of this nature was first seen by Langley & Kato (1914), who found the central convulsant action of eserine was diminished by atropine, and similar actions of atropine have been observed subsequently in poisoning by DFP (Modell & Krop, 1946) and other alkyl phosphate anticholinesterases (Gilman, 1946). Some workers, however, have found atropine ineffective against the convulsant action of DFP (Heymans & Jacob, 1947) and TEPP (Burgen, Keele & Slome, 1949), but the discrepancy may be due to difference in dosage. Heymans & Jacob do not state the amounts used, but Burgen *et al.* certainly used less atropine than we have done. In our experiments fairly large doses of atropine (or hyoscine), such as were required to restore the respiratory centre, were found necessary to diminish convulsions, whereas small doses of atropine sufficient to control the parasympathomimetic effects had little

apparent central action. In favour of this interpretation is the observation of Wescoe, Green, McNamara & Krop (1948) that the convulsive electroencephalogram patterns induced by DFP were controlled by atropine or hyoscine, but only in large doses (1 mg./kg. or more).

The presence of a significant central component in the action of atropine, such as we have found in TEPP poisoning, renders it difficult to interpret experiments which employ atropine as a pharmacological tool to analyse the mechanism of death from anticholinesterase poisoning. Modell & Krop's (1946) assumption that death from DFP in the cat was largely muscarinic because it was relieved by atropine is a case in point. Furthermore, atropine treatment of anticholinesterase poisoning should take into consideration the control of central as well as parasympathomimetic symptoms, and dosage should be regulated accordingly. Clinical experience which, from the beginning, has demonstrated the usefulness of atropine in controlling parasympathomimetic actions, is providing growing evidence of atropine antagonism to the central effects. Grob *et al.* (1950), for example, found that atropine restored consciousness in patients comatose as a result of exposure to the anticholinesterase Parathion. At the same time, the doses of atropine they gave to their patients, although large by usual clinical standards, are small by comparison with the amounts needed to control central effects in the cat. Death in these patients, as in the cat, seems to be largely due to depression of medullary centres, and higher doses of atropine (or indeed of hyoscine) might well prove beneficial.

It is generally held that TEPP inhibits cholinesterase in an irreversible manner, and certainly no recovery of activity is apparent when reversibility is sought *in vitro* by dilution techniques (Brauer, 1948; Augustinsson & Nachmansohn, 1949; Aldridge, 1950). It may therefore seem surprising that the toxic effects of TEPP we have described are of such short duration. Doubt may even arise as to whether the symptoms are, in fact, attributable to cholinesterase depletion. An answer has been provided by the demonstration that the inhibition of cholinesterase caused by TEPP *in vivo* is reversible, at least in part, and that recovery of tissue cholinesterase activity is much more rapid than after DFP (Dayrit, Manry & Seevers, 1948; Hobbiger, 1951). Subsequent experiments have shown that reversibility can also be demonstrated *in vitro*, provided the TEPP-poisoned tissue preparations are incubated over a sufficient length of time (Grob & Harvey, 1949; Hobbiger, 1951). Whatever the explanation of this reversibility (see Burgen, 1949; Hobbiger, 1951), it supports the simple view that the rapid recovery from TEPP poisoning is brought about by quick restoration of cholinesterase activity.

SUMMARY

1. The cause of death from intravenous injection of tetraethylpyrophosphate (TEPP) was examined in cats.

2. Injection of 0·3–0·6 mg./kg. caused death by respiratory failure. The factors responsible for this were paralysis of the respiratory muscles and failure of the respiratory centre. Bronchoconstriction occurred but played a secondary role.

3. Paralysis of the respiratory muscles was due to neuromuscular block. The block showed the characteristic features of cholinesterase inhibition and probably resulted entirely from acetylcholine accumulation at the end-plate region.

4. Failure of the respiratory centre—observed by recording the action potentials in the phrenic nerve—was independent of the peripheral actions of TEPP on lung ventilation and blood pressure. It appeared to be due to an action of TEPP within the central nervous system.

5. The effects of TEPP on the neuromuscular junction and respiratory centre were reversible. Neuromuscular block lasted some 15 min.; central respiratory failure was more persistent.

6. The neuromuscular block was partly relieved by pentamethonium (C5).

7. Failure of the respiratory centre was prevented or overcome by atropine or hyoscine.

8. Atropine or hyoscine diminished the central convulsions which were observed after TEPP, and in addition controlled the parasympathomimetic effects. Thus, provided the transient paralysis of the respiratory muscles was combated by artificial respiration, these alkaloids prevented death even from large doses of TEPP.

REFERENCES

Aldridge, W. N. (1950). *Biochem. J.* **46**, 451.
Augustinsson, K.-B. & Nachmansohn, D. (1949). *J. biol. Chem.* **179**, 543.
Bacq, Z. M. & Brown, G. L. (1937). *J. Physiol.* **89**, 45.
Brauer, R. W. (1948). *J. Pharmacol.* **92**, 162.
Burgen, A. S. V. (1949). *Brit. J. Pharmacol.* **4**, 219.
Burgen, A. S. V., Keele, C. A. & Slome, D. (1949). *J. Pharmacol.* **96**, 396.
de Candole, C. A., Douglas, W. W. & Spencer, K. E. V. (1950*a*). *J. Physiol.* **111**, 21*P*.
de Candole, C. A., Douglas, W. W. & Spencer, K. E. V. (1950*b*). *J. Physiol.* **111**, 23*P*.
Chennells, M., Floyd, W. F. & Wright, S. (1949). *J. Physiol.* **108**, 375.
Dayrit, C., Manry, C. H. & Seevers, M. H. (1948). *J. Pharmacol.* **92**, 173.
Douglas, W. W. & Matthews, P. B. C. (1951). *J. Physiol.* **114**, 31*P*.
Fatt, P. & Katz, B. (1950). *Nature, Lond.*, **166**, 597.
Feldberg, W. (1945*a*). *J. Physiol.* **103**, 367.
Feldberg, W. (1945*b*). *Physiol. Rev.* **25**, 596.
Gesell, R. & Hansen, E. T. (1943). *Amer. J. Physiol.* **139**, 371.
Gilman, A. (1946). *Ann. N.Y. Acad. Sci.* **47**, 549.

Gilman, A. & Koelle, G. B. (1949). *J. Pharmacol.* **95**, 166.
Grob, D., Garlick, W. L. & Harvey, A. M. (1950). *Johns Hopk. Hosp. Bull.* **87**, 95.
Grob, D. & Harvey, A. M. (1949). *Johns Hopk. Hosp. Bull.* **84**, 532.
Heymans, C. & Jacob, J. (1947). *Arch. int. Pharmacodyn.* **74**, 233.
Hobbiger, F. (1951). *Brit. J. Pharmacol.* **6**, 21.
Langley, J. N. & Kato, T. (1914). *J. Physiol.* **49**, 410.
Lundholm, L. (1949). *Acta physiol. Scand.* **16**, 345.
Miller, F. R. (1949). *Canad. J. Res.* **6**, 374.
Modell, W. & Krop, S. (1946). *J. Pharmacol.* **88**, 34.
Paton, W. D. M. & Zaimis, E. J. (1949). *J. Physiol.* **108**, 55*P*.
Schweitzer, A. & Wright, S. (1938). *Quart. J. exp. Physiol.* **28**, 33.
Verbeke, R. (1949). *Arch. int. Pharmacodyn.* **80**, 19.
Wescoe, W. C., Green, R. E., McNamara, B. P. & Krop, S. (1948). *J. Pharmacol.* **92**, 63.
Wilson, K. M. (1950). *J. Physiol.* **111**, 31*P*.
Wood, J. R. (1950). *J. Amer. med. Ass.* **144**, 606.