STUDY OF ANALEPTICS

by

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Contents.

I. INTRODUCTION.

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The improvement of lowered conditions of vitality brought on by disease or poisoning must have been a problem engaging the serious attention of people practising the healing art from the earliest days of human civilisation. This led to the use of various agencies, supposed to possess reviving powers, some of which survived through long periods of usage. Therapeutics in early days was purely empirical. Even after considerable advances in the knowledge of the pathology of these lowered conditions of vitality, very little attention was paid to enquiring into the nature of the reviving power of these agencies(by which was perhaps meant the improvement of some symptoms of lowered conditions). In many cases the improvement was due to the strong local irritant action of alcohol and other irritant constituents of the medicaments on the gastric mucosa, setting up a strong reflex stimulation of the respiratory and vasomotor centres and also perhaps partly to alcoholic contents thereof, acting as a diffusible stimulant/

stimulant, and supplying ready nutrition to tissues. A few did act after absorption, and some of these caused improvements in lowered conditions by improving the circulation as cardio-vascular stimulants and a few others really acted through the nervous system, especially through the respiratory and other vital centres.

Development of modern surgery brought in its train the use of anaesthetics which have their risks of extreme depression. This inspired an increased study of the nature of reviving drugs. With the rapid increase in the use of barbiturates as basal narcotics and anaesthetics, and also with the occurrence of poisoning, following their therapeutic administration, self-medication or suicidal attempts, the study of analeptic drugs has, in recent years, been engaging a good deal of attention of pharmacologists. Of the older drugs, picrotoxin and strychnine have been very thoroughly investigated, as also coramine and cardiazol amongst the newer synthetic drugs. Caffeine, cocaine, ephedrine, lobeline and a few others have also received some attention.

The clinical use of some of these still persists in spite of their questionable status

as/

as judged by experimental evidences.

No doubt a good number of therapeutic agents are of more or less symptomatic value in combating some of the effects of hypnotics and narcotics. But there is still a good deal of lack of unanimity regarding their usefulness in different stages of depression produced by the narcotics. The importance of the subject therefore called for further study of the analeptics.

Moreover, methods employed for the study of the problem were diverse in nature, and most of them had some unsatisfactory feature or other. For a truer evaluation and determination of the comparative worth of these drugs it was found necessary to evolve an efficient method free from those unsatisfactory features. Investigations undertaken for these purposes form the subject of this thesis.

II. /

II. RESUMÉ OF ANALEPTIC LITERATURE.

(A) Pharmacological.

(B) Clinical.

(A) Pharmacological.

Picrotoxin.

Picrotoxin was first isolated in 1812 from Fish Berry - Cocculus Indicus - for a long time known to be used for poisoning fish. (The fish rose to the surface gasping, on account of their swim-bladder being distended with air. This rendered such fish poisonous). A similar substance also used to be added to beer to increase the bitter taste and this resulted in cases of poisoning. Picrotoxin is a non-alkaloidal non-nitrogenous neutral substance. Its structural constitution is not yet definitely established, the chemical composition being represented by $C_{30}H_{34}O_{13}$. Cervello (1911) and Hormann (1913) found it to consist of two components, picrotinin $C_{15}H_{16}O_6$, the pharmacologically active part, in feeble combination with a non-active component, picrotin, $C_{15}H_{18}O_7$.

The first publication in analeptic literature comes from Tschudi (1847), who found picrotoxin antagonistic in action to morphine. Crichton Brown (1875) working on rabbits and guinea pigs, observed a physiological antagonism between picrotoxin/

picrotoxin and chloral hydrate as chloral could control picrotoxin convulsion and picrotoxin raised the lethal dose of chloral from 0.5 gr. to 1 gr. per kilo. Amagat (1876) confirmed Brown's findings. (Perrier (1875) observed that in frogs and rabbits, toxiresins produced picrotoxin-like convulsions which could be controlled with chloral hydrate). Luchsinger (1878) reported that tetanic convulsions of picrotoxin persisted even after the destruction of the medulla. Gottlieb (1892) obtained similar results and also reported that picrotoxin could interrupt the narcosis produced by paraldehyde and weaken that of the alcohol group of narcotics. Maxwell (1906) reported that the direct application of picrotoxin to the cerebral motor cortex caused some stimulation, whereas similar application to the white matter produced none.

Greenwald (1909) and Morita (1915a) found that picrotoxin convulsions were not influenced by destruction of the hemispheres. Pollock and Holmes (1915) observed that picrotoxin convulsions were not influenced by section below the optic thalamus. Janusche (1918) found that picrotoxin awakened/

awakened guinea pigs from bromide sleep. Maloney and co-workers (1931, 1932) found that picrotoxin, even in small doses, given immediately before or after barbiturates, either prevented narcosis or shortened recovery period and saved the life of the animals which had received more than the usual fatal dose. They further found that the lowered body temperature caused by barbiturate depression was quickly brought back to normal by picrotoxin which also caused a prolonged rise of arterial pressure and fall of venous pressure and stimulated the respiratory mechanism. Swanson (1932) reported detoxication of picrotoxin, strychnine and cocaine by amytal. Gower and Erve (1933) reported that picrotoxin saved the life of dogs after one and a half lethal doses of barbital sodium. Barlow (1935) studied the shortening of the period of recovery from barbiturate hypnosis produced by different analeptics and also observed the effects on respiration, circulation and reflex excitability and judging by the degree of improvement in all these, estimated the relative efficiency and degree of usefulness to be in the order of picrotoxin, metrazol, ephedrine, coramine, strychnine and caffeine/

caffeine. Koppanyi et al. (1936) found that 4 M.L.D. of barbital were counteracted by picrotoxin and that 70 M.L.D. of picrotoxin could be counteracted by barbital. Kranz, Car and Beck (1937) found that picrotoxin antagonised the depression of oxygen consumption produced by nembutal. Marshall, Walzl and LeMessurier (1937) observed that in barbiturate narcosis picrotoxin stimulated the depressed respiration by antagonising the depressants, whereas in normal animals, no stimulation of respiration occurred except after convulsive doses. Kohn (1938) determined the median lethal dose of picrotoxin for rabbits to be between 1.2 and 1.3 mg. per kilo. and found that the M.L.D. rose to 7.5 mg. after nembutal. Repeating sublethal doses of picrotoxin for several days, he found no evidence of tolerance for the drug.

Strychnine, $C_{21}H_{22}N_2O_2$.

Strychnine was isolated in 1818 by Pelletier and Caventou. Arzt Dresbach (1850) reported that he observed some degree of antagonism between strychnine and chloroform. Liebreich (1870) noted that chloral could not prevent symptoms of and death from strychnine. Arnould (1870) and

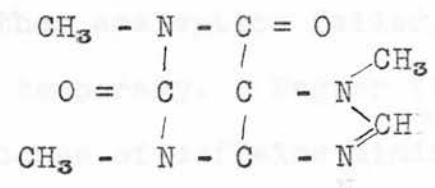
Ore/

Ore (1872) could not get any beneficial effect from strychnine in chloral poisoning. Rajewasky (1872) reported to have succeeded in antagonising strychnine with chloral, provided the dose of strychnine was not too large. Hager and Steinon (1875) reported strychnine to be ineffective against chloral poisoning. Husemann(1879) tried chloral against strychnine, brucine and physostigmine and reported that chloral modified the excitement of these drugs by depression of the cord but the reverse was not true as the spinal excitants could not neutralise the coma of narcotics produced by the action on higher centres. Cushny (1913) found that in animals, therapeutics doses of strychnine increased the rate of respiration. Lovenhart (1918) reported that strychnine produced improvement in depression of respiration caused by cerebral compression. Hanzlik (1923) found that strychnine caused acceleration of respiration when excitability was partly depressed, especially in anaesthesia and morphine poisoning. Dragstead and Lang (1928), and Dawson and Taft (1931) reported experimental proof of efficiency of barbiturates/

barbiturates in strychnine poisoning. Maloney et al. (1931) reported antidotal value of strychnine against barbiturates to be quite low. Haggard and Greenberg (1932) reported the antagonism between strychnine and pentobarbital. Barlow (1932) stated that strychnine and pentobarbital were mutually antagonistic within limits, but strychnine in large doses further depressed respiratory mechanism already rendered hypoactive by the hypnotic. Chauchard (1934) demonstrated that strychnine augmented chronaxae diminished by barbiturates. Maloney (1935) found that in cases of severe depression of respiration by barbiturates, strychnine and cocaine hastened respiratory failure. Barlow (1935) placed strychnine between picrotoxin and metrazol as a resuscitation agent in order of effectiveness in pentobarbital poisoning, but considering the margin of safety and improvement of respiration and circulation, placed strychnine below picrotoxin. Metrazol, ephedrine and coramine.

Caffeine/

Caffeine, $C_8H_{10}ON_4O_2$



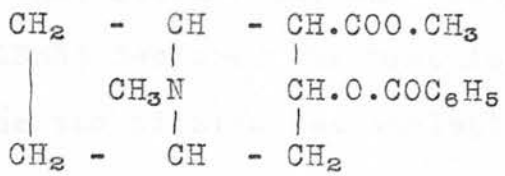
1.3.7-trimethyl-2.6.dihydroxy purine.

Caffeine was isolated in 1820 by Runge, Pelletier and Caventou. Bennett (1874) used caffeine against morphine poisoning. Binz (1878) found that caffeine definitely antagonised alcohol in dogs and that caffeine increased the respiratory rate in anaesthetised animals. Heinz (1890) reported that he obtained an increase in depth of respiration after caffeine but Cushny (1913) found that in decerebrate animals, caffeine only increased the rate of respiration and depth either remained unchanged or even became shallower. Airilla (1913) observed that caffeine was only of temporary value against chloral. Vogtlin and Wiggers (1918) found that caffeine slightly stimulated respiration and circulation in depressant poisoning but the effects soon became additive to the depressants. Hanzlik (1923) found that the respiration of unanaesthetised animals was depressed by 10 mg./kg. doses of caffeine/

caffeine. Duke (1923) reported that in narcotic poisoning, caffeine restored consciousness even when other analeptics failed, but the effect was merely temporary. Wagner (1931) reported that small doses of caffeine diminished the depth of light hypnosis of chloral but large doses exaggerated the depressant effects of chloral. Barlow (1935) found that caffeine in good doses only moderately improved the respiration after hypnotics, and excessive doses, after the initial stimulation, had a secondary depressant action which was additive to that of the hypnotics. Maloney (1935) reported that sub-lethal doses of caffeine after single sub-lethal barbiturate dosage in rats, caused transient arousal followed by relapse, and beyond a stage added to the depression of the hypnotic and killed by depression and not by convulsion and declared it as contraindicated in deep barbiturate depression.

Cocaine/

Cocaine, $C_{17}H_{21}NO_4$.



Cocaine was isolated in 1860 by Niemann. Mosso (1887) reported diminution of depth of chloral narcosis by cocaine. Feinberg and Blumenthal (1887) reported absence of cocaine convulsion after ablation of the cerebrum in dogs. Morita (1915) found in rabbits, deprived of cerebral hemispheres, absence of clonic but onset of tonic convulsion and increase in resistance to toxic doses. Airila (1913) found dogs awakened from deep chloral sleep after cocaine. Sollmann (1932) states that stage of stimulation may be short or absent, succeeded with larger doses by depression of co-ordinating centres and that seat of convulsive action is descending, different centres being affected in succession. Roth (1917) found that small doses increased respiration while large doses were depressant, killing by respiratory paralysis. Tatum, Atkinson and Collins (1925) found that barbital controlled cocaine convulsion, but the reverse was found not true/

true. Swanson (1932) stated that cocaine antidoted lethal doses of barbiturates, but Maloney (1933) reported that cocaine hastened respiratory failure. Barlow (1935) declared the practical usefulness of cocaine and nicotine as analeptics to be questionable.

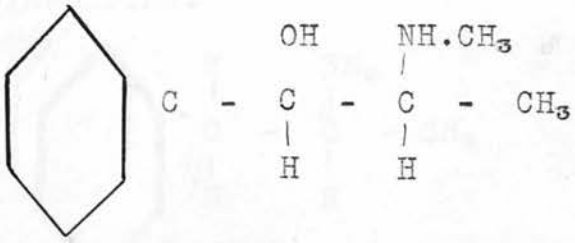
Lobeline, $C_{16}H_{24}NO$.

Lobelia is a native plant of North America, where it has been for centuries used as a remedy for bronchitis and asthma. It was introduced into medicine about the beginning of the nineteenth century by Samuel Thompson who used it against morphine to stimulate respiration. It contains a liquid alkaloid $C_{16}H_{24}NO$. Small doses of it were stated by Sollmann to lower CO_2 threshold producing marked stimulation of respiration. He further stated it to be effective in overcoming respiratory depression of morphine in the rabbit, and less effective in chloral depression, but the response was uncertain. Eckstein (1921) considered it clinically valuable in pneumonia and asphyxia. Norris and Weiss (1927) stated it to be ineffective and even dangerous. Wieland and Mayer (1927) experimentally produced lowered CO_2 threshold and stimulation/

stimulation of respiration by lobeline. This was less constant or absent in morphinised animals and effective doses produced toxic ~~morphinised~~ effects and secondary depression.

Ephedrine, $C_{10}H_{15}NO$.

The Chinese plant "Ma Huang" was known and used there as a therapeutic agent over 2000 years. Its active alkaloid ephedrine is pharmacologically

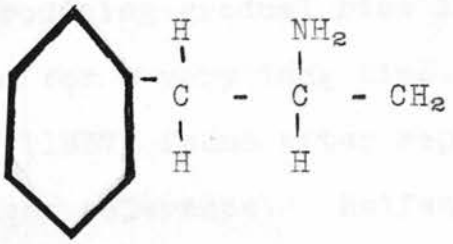


similar to adrenaline and is more stable but many times weaker so that proportionately bigger doses produce slower and more prolonged effects. Airilla (1913) reported that ephedrine antagonised effects of chloral hydrate. Morita (1915b) confirmed this. Kreitmair (1927) found that ephedrine antagonised morphine and morphine-scopolamine depression. Rajewsky and Bourne (1931) reported that ephedrine diminished or interrupted avertin narcosis. Barlow (1935) stated that from degree of symptomatic improvement, ephedrine appeared very effective against chloral because of circulatory stimulation, but/

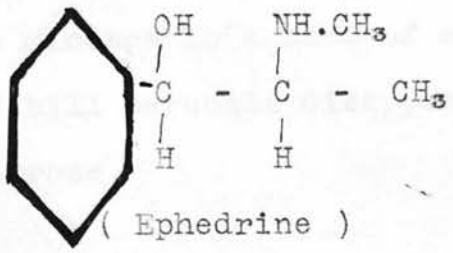
but large doses tended to give added depression, and that medullary and vasopressor effects of ephedrine markedly accentuated the stimulant effects of picrotoxin, metrazol and coramine.

Benzedrine.

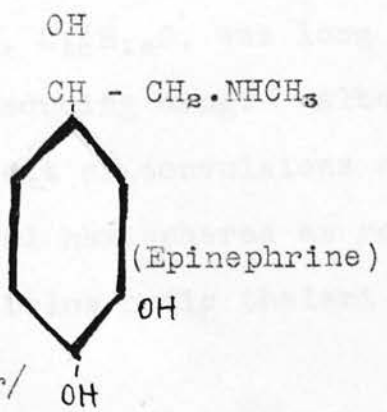
Barger and Dale (1910) first described benzedrine as β-phenyl-isopropylamine. It is a sympathomimetic drug closely related to ephedrine and epinephrine.



(Benzedrine)



(Ephedrine)



(Epinephrine)

Tainter (1933) and Alles (1933) reported on its central stimulating effects. Prinzmetal and Blooomberg (1935) stated that benzedrine possessed more stimulating effect on the central nervous system than ephedrine and was able to awaken animals from experimental barbiturate narcosis and also showed an insomnia-producing effect on human cases. They also reported successful cases of narcolepsy treated with 10-40 mg. Mayerson, Loman and Dameshek (1936) reported of its producing gradual rise in blood pressure sustained for a very long time. Ehrich and Krumbahr (1937) found after repeated doses in rats very slight tolerance. Reifenstein and Davidoff (1938) reported using 10 mg. of benzedrine sulphate every 15 minutes in a case of amytal sodium poisoning (0.5 g.) till narcosis disappeared and blood pressure rose.

Camphor and Allied Drugs.

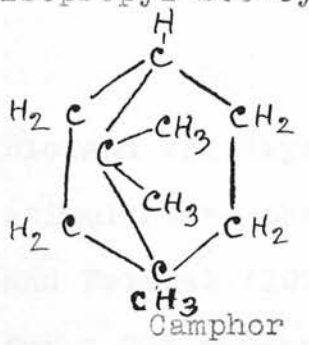
Camphor, $C_{10}H_{16}O$, was long ago known to be a convulsion-producing drug. Alberton (1882) located the seat of convulsions of camphor to be in the cerebral hemispheres as removal of these with section below optic thalami diminished or abolished/

abolished them. This was confirmed by Morita (1915). Gottlieb (1892) reported that camphor antagonised or interrupted paraldehyde hypnosis Sollmann stated that effects of non-convulsive doses of camphor on respiration were slightly inconstant and frequently negative. Wieland(1915) reported lowering of CO_2 threshold. Hanzlik (1923) reported fair increase of respiration after hypodermic injection of camphorated oil into animals. Camphor was also reputed to be a circulatory stimulant although various workers reported contradictory results as to its effectiveness and limitations. Very poor solubility of camphor in water and very slow absorption from oily solution injected (allowing camphor to be converted into non-active campho-glycuronic acid and preventing effective concentration in blood) together with continental faith in efficacy of camphor, led to introduction of a series of camphor substitutes, notably hexatone, coramine and cardiazol.

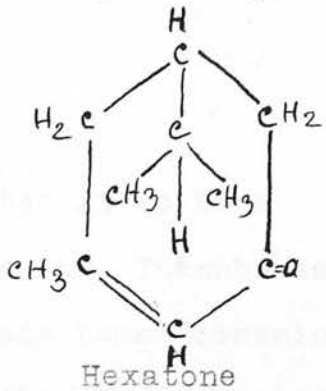
Hexatone.

Hexatone.

This is a camphor isomer being 3-methyl-1.5-isopropyl-2.3-cyclohexanone.

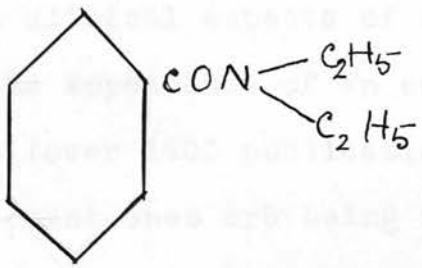


Camphor



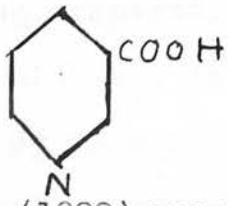
Hexatone

This is also not very soluble in water but is soluble in a 20% solution of sodium salicylate. Part of the effect obtained from this was undoubtedly due to the solvent and hexatone failed to fulfil the expectations that created it.

Coramine. 

This is a thick yellowish liquid, freely soluble in water and other organic solvents and fairly stable. It is not irritant locally or subcutaneously and absorbed pretty rapidly from oral, subcutaneous or intramuscular injection.

The first published reference to this drug came in 1922 from Lagier who discussed its pharmacological properties as a circulatory stimulant under the name "nicotinic acid-diethyl-amide/

amide" from its structural resemblance with nicotinic acid 

Wieland and Mayer (1922) reported it to be a stimulant to the respiratory centre. Thannhauser and Fritzel (1924) used the trade name "coramine" for a 25% aqueous solution of the drug and stated this to be of definite value in collapse and cardiac decompensation. Faust (1925) described it as pyridine-β-carboxy-diethylamide and reported it to be a cardiac, respiratory and vasomotor stimulant. Since the introduction of this drug, the experimental and clinical aspects of its activity have provoked the appearance of an enormous amount of literature (over 1400 publications) out of which a few important ones are being referred to.

Uhlmann(1924), Asher (1928), Schübel and Gehlen (1928) confirmed the previous reports of Wieland and Mayer about its having a good respiratory stimulant action. Helaers (1924) also supported this and declared it to be very prompt and reliable. Earlier reports of Lagier about its circulatory stimulant action were confirmed by Asher (1925), Burgi and Gordonoff and many other workers. Leyko (1930), employing

Starling/

Starling heart-lung preparation reported increase in coronary flow which was later confirmed by Mezey (1935) and by Greene (1936). Zunz and Trimonti (1931) employing denervated carotid sinus, showed that coramine lost its respiratory stimulant action after morphine depression, whereas cardiazol gave prolonged stimulation, and concluded that coramine stimulated respiratory centre indirectly through carotid sinus in normal therapeutic doses and directly only after large doses. Schoen (1926) reported light narcosis of paraldehyde (1 g./kg.) and of chloral (0.6 g./kg.) controlled by 50 mg./kg. of coramine in 10 minutes, but large or repeated small doses could not control deep narcosis. Mörl (1931), Killian and Uhlman (1931) and several others report favourably in tribrom-methanol depression, but Jager (1932) and Lendle (1936) found narcosis prolonged, and Albus (1932) reported that awakening doses of coramine were highly toxic and killed. Tartler (1929) found only transient awakening after 200 mg./kg. of medinal in rats amd the smallest effective dose caused appearance of cramp, and larger doses

while/

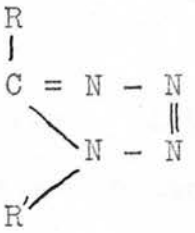
while producing clonic spasms drove animals into fuller narcosis. Maloney, Fitch and Tatum (1931) found general unsuitability of coramine in barbiturate poisoning. Moritch (1932) found that after veronal, luminal and somnifene, the sleep deepened and the toxicity increased by coramine. Kohn and Jacobi (1935) and Zipf (1936) also reported fuller narcosis with the drug and also stated that it hastened death of experimental animals. Lendle (1936) found pernocton narcosis often lengthened by coramine. Zipf and co-workers (1937) observed prolonging of narcosis from pernocton, rectidon and eunarcon. Schwab and Jung (1936) found in guinea pigs under evipan narcosis, recovery greatly prolonged (from 2 or 3 hours to about 20 hours). Zipf (1937) reported similar findings in rats. Whitehead and Draper (1939) used a closed soda-lime absorption system for inhalation of volatile anaesthetics and determined the minimal dose of ether and chloroform required for the respiratory arrest of individual dogs (which were later on resuscitated by oxygen and artificial respiration). They found the intravenous injection of 37.5 mg./kg. of coramine did not cause any change in the minimal amount required for the respiratory/

respiratory arrest in either case and in the case of chloroform doubled the rate of mortality.

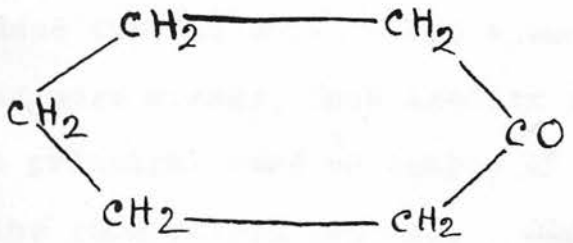
Cardiazol.

Cardiazol is a white crystalline solid having a sweetish aromatic odour, readily soluble in water and saline, making a neutral solution which can be sterilised by boiling. It is non-irritant to skin and mucous surfaces and is very readily absorbed even after oral administration and there is little difference in reaction time following intravenous injection as compared to hypodermic route.

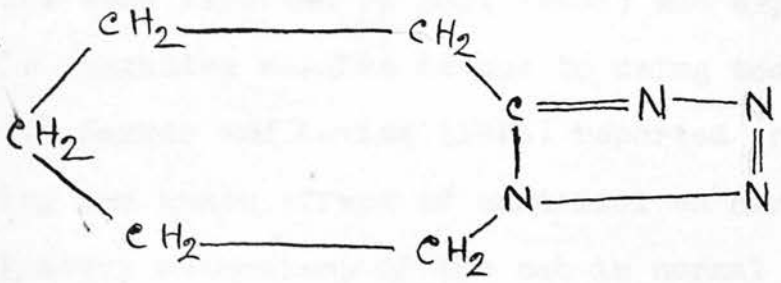
In 1924 Schmidt discovered the method of catalytic splitting of hydrazoic acid HN_3 into HN and N_2. Later he succeeded in introducing tetrazole radicle $= N - \overset{\|}{N} - N = N$ (obtained by oxidation of two HN_3 groups) into nitrogen-free carbonyl compounds (of the type $\begin{matrix} R \\ \diagdown \\ C = O \\ \diagup \\ R' \end{matrix}$, thus forming bicyclic tetrazole compounds/

compounds of the type 

The next step was the breaking into the cyclohexanone ring for the introduction into it of a



nitrogen atom of the tetrazole radicle, thus yielding pentamethylene tetrazole, which was given the trade name cardiazol (or metrazol in U.S.A.).



The first report of the investigation of this drug came from Krehl (1925) and then from Schmidt, Hildebrandt and Krehl (1925). Later Hildebrandt made more through study of the drug and reported it to be a stimulant to heart and central nervous system, especially the respiratory centre. The respiratory stimulant action was later confirmed/

confirmed by various workers. Schoen (1926) studied the seat of convulsion produced in rabbits by extirpating cerebral hemispheres which produced no change, and on further removal of thalami, basal ganglia, and anterior quadrate bodies, could still produce typical convulsions although with three times more dosage, thus leading to the conclusion that principal seat of action of cardiazol was in the subcortical centres. Camp (1928) could not find any increase of spinal irritability in rats and rabbits after cardiazol. Contrary results were reported by Koll (1937) who explained Camp's negative results as due to using too small doses. Barker and Levine (1928) reported not finding any tonic effect of cardiazol on cardio-respiratory mechanisms of the cat in normal or depressed condition. Watt (1929) found it depressing mammalian heart and showing no pressor action in deep narcosis. Johnston (1929) found clinical proof of usefulness of cardiazol in circulatory collapse and myocardial insufficiency. Leyko (1930) using Starling heart-lung preparation, found/

found after cardiazol no effect on tone of heart or coronary circulation. Orestano (1929) attributed respiratory improvement to cardiac action whereas Gremels (1931) held circulatory improvement as secondary to respiration and dependant upon it. Buding (1930) classed cardiazol as a picrotoxin-like drug; Schwab and Guizetti (1931) placed cardiazol between digitalis and camphor as circulatory stimulant. Schoen (1926) reported awakening by intravenous cardiazol in urethane, alcohol and paraldehyde narcosis. Tartler (1929) and Mehl (1930) found medinal sleep of rats broken by cardiazol. Medinal-cardiazol antagonism was also reported by Maloney (1935) and Kohn and Jacobi (1936). Jager (1932) reported awakening from avertin sleep by cardiazol. Kohn and Jacobi (1935) Lendle (1936), Albus (1936) and Zipf and Mertins (1937) reported similar results. Barlow (1935) Gros (1936, Zipf et al. (1937) found cardiazol antagonising chloral hydrate and Schoen (1926), Gros (1936), Hoffmann (1936), paraldehyde. Schwab and Jung (1936) found evipan narcosis shortened to half by cardiazol and convulsive and lethal doses of/

of cardiazol increased by 2 to 3 times after evipan. Steininger and Gaubatz (1935) reported cardiazol better than coramine in morphine poisoning. (1935) Barlow placed cardiazol as next to picrotoxin as a reviving drug and Koppanyi et al. (1936) described it as equal to or better than picrotoxin as awakening agent. Thiel (1937) found in morphine plus pernocton narcosis, in dogs, cardiazol-sympathol combination better than coramine or lobeline; whereas in CO poisoning neither cardiazol nor coramine had any effect. Rice and Isenberger (1937) had no improvement in amytal narcosis by intracisternal injection of cardiazol or coramine whereas picrotoxin reduced the duration of respiratory paralysis. Werner (1938) in a comparative study of picrotoxin, cardiazol and coramine, placed cardiazol as next to picrotoxin in order of efficiency.

B. /

(B) Clinical.

In 1879 Murrel reported (in The Practitioner) having used picrotoxin clinically in 0.5 to 1 mg. doses. Further reports appeared in the British Medical Journal of 1880. Howard (1890) reported that he used with advantage 0.6 mg. for children and 1.2 mg. for adults. Strychnine has also been used by various clinicians in earlier days. With rapid increase in accidental and suicidal cases of narcotic poisoning and consequent extensive experimental investigations, therapeutic uses of analeptics against narcotics and vice versa have received a fresh impetus in recent times. Thus picrotoxin has received considerable attention in America, whereas the French school has taken to strychnine, and in Germany, camphor derivatives particularly coramine and cardiazol had extensive clinical application. Reports about the last group are numerous and varied; various people reported about them as having yielded excellent results as respiratory and even circulatory stimulants, and some others gave less favourable reports and a few reported of having disappointing and even contrary results. For example Killian (1931), Morl (1931), Domanig (1931), Fischman/

Fischmann (1932), Kennedy (1932), Specht (1932) reported having very encouraging results from coramine in avertin depression. Glaesser(1933) reported success in luminal poisoning, Altmann (1932) in barbital poisoning (270 g.), Ludwig and Hortnagel (1932) in opium poisoning and Wood (1933) in surgical shock. Jager (1933) on the other hand, reported failure of coramine to influence respiratory depression.

Report of clinical use of strychnine as analeptic in recent times comes from Ide (1932) who stated having good results in veronal poisoning in man. Luschke (1933) reported similar clinical experience and recommended big and frequently repeated doses. Kouman (1934) reported cure of a case of strychnine poisoning by intravenous pernocton and Hamori (1936) of a case of pernocton poisoning by strychnine. D'Oelsnitz and co-workers (1933) reported two severe cases of barbiturate coma treated with strychnine, one of which survived and the other died in spite of strychnine. Thereon these authors stated that individual sensitivity differed and that some cases led/

led to more quick fixing of barbiturate in the bulbar centres than others. Flandin and Bernard (1933) reported death of a case of barbiturate poisoning after 67 hours of strychnine treatment. Von Paraf, Delay and Macrez (1933), and Ramon and Delay (1933) similarly reported death in spite of strychnine. Olmer and Audier (1934) state that the result depends on the intensity of barbiturate poisoning and recommend in every case trial with strychnine.

Burstein and Rovenstein (1937) reported on clinical use of picrotoxin, metrazol (cardiazol) and coramine, recommending 3 mg. of picrotoxin i.v., repeated in 5 minutes and more if necessary till symptomatic effect produced and then intramuscularly; metrazol 1.5 to 2 c.c. intravenously repeated in 5 minutes and then intramuscularly, and coramine 25 c.c. or repeated doses of 5 c.c. till 25 c.c. given. They found 3 to 6 mg. of picrotoxin giving best response (improvement of rhythmicity, depth and rate of respiration and frequently stimulation of circulation) and causing less prominent cerebral symptoms than metrazol in morphine depression. Intravenous metrazol caused immediate improvement in/

in respiration and circulation, but the effect was transitory. Coramine effect was equally transitory and less marked. In ether, paraldehyde and cyclopropane depression metrazol effect was best and coramine least. Only in tribrom-methanol depression coramine was most effective. In depression of spinal anaesthesia, fall of blood pressure and respiratory minute volume were not appreciably relieved by any, whereas circulatory depression of surgical shock was not materially benefited by any one alone.

Bleckwenn, Masten and Tatum (1937) reported having found picrotoxin as best of all in barbiturate poisoning. They used 1 in 1000 solution, 1 c.c. per minute, i.e. 1 mg. per minute intravenously.

Rovenstein (1938) reported four severe cases of barbiturate poisoning treated by picrotoxin of which only the last one could not be saved. The first (114 g. amytal sodium) received 3 mg. doses of picrotoxin till 129 mg. given, the second (90 g. nembutal) had 3 mg. doses till 48 mg. The third (75 g. of sodium luminal) had 128 mg. and the fourth (500 g. of veronal) had 23mg. in the beginning/

beginning (at 1 mg. per minute), then 24 mg., 39 mg., 21 mg. and 23 at intervals of one to two hours, along with intravenous saline, and then 20 mg. per hour intramuscularly for 20 hours, and finally 10 mg. per hour intramuscularly. The case appeared practically recovered and treatment was stopped but suddenly symptoms relapsed ending in death. From his clinical experience, this author advises that besides persistent treatment with picrotoxin, one must attend to other therapeutic measures like maintenance of body temperature, frequent gastric lavage, catheterisation, frequent clearing of trachea and change of posture, besides diuretics, oxygen and even blood transfusion.

III. PRODUCTION AND MAINTENANCE OF A STEADY STATE OF RESPIRATORY DEPRESSION BY CONTINUOUS INFUSION OF SODIUM EVIPAN.

The chief purpose for which analeptics are used clinically is the stimulation of the respiratory centre, and the most promising method for investigating the action of analeptics is to measure their effect on the respiratory centre after this has been depressed. The chief difficulty is to obtain a constant degree of depression of the respiratory centre. One method of producing this effect is to administer some drug, such as phenobarbitone, which has a very prolonged action. The defence mechanisms of the body against drugs are however so complex and efficient, that it is difficult to obtain a prolonged and uniform depression by such means.

An alternative method is to give a continuous intravenous infusion of a drug which is rapidly destroyed by the body. Sodium evipan is a familiar example of such a drug, and experiments were therefore made to determine whether it was possible to produce a steady degree of respiratory depression by means of continuous intravenous infusion/

infusion of evipan.

Method.

The respiration was measured by means of a plethysmograph which was a modification of that used by Cushny (1913) for investigating the pharmacology of the respiratory centre.

Fig. 1 gives a diagrammatic sketch of the apparatus and arrangements. A lead sheet, 16" x 12" x $\frac{1}{8}$" was bent along its shorter axis into the shape of a dome. The animal (rabbit) was placed on the table on its back. The dome, when placed over the animal, remained quite clear of its body. An airtight bag, made out of very thin rubber cloth, was interposed between the dome and the animal's body. The bag was broad enough to cover the animal's body from side to side and extended over the whole of the animal's chest and abdomen. When properly inflated, the bag occupied the whole space between the dome and the animal's body.

Fig. 1/

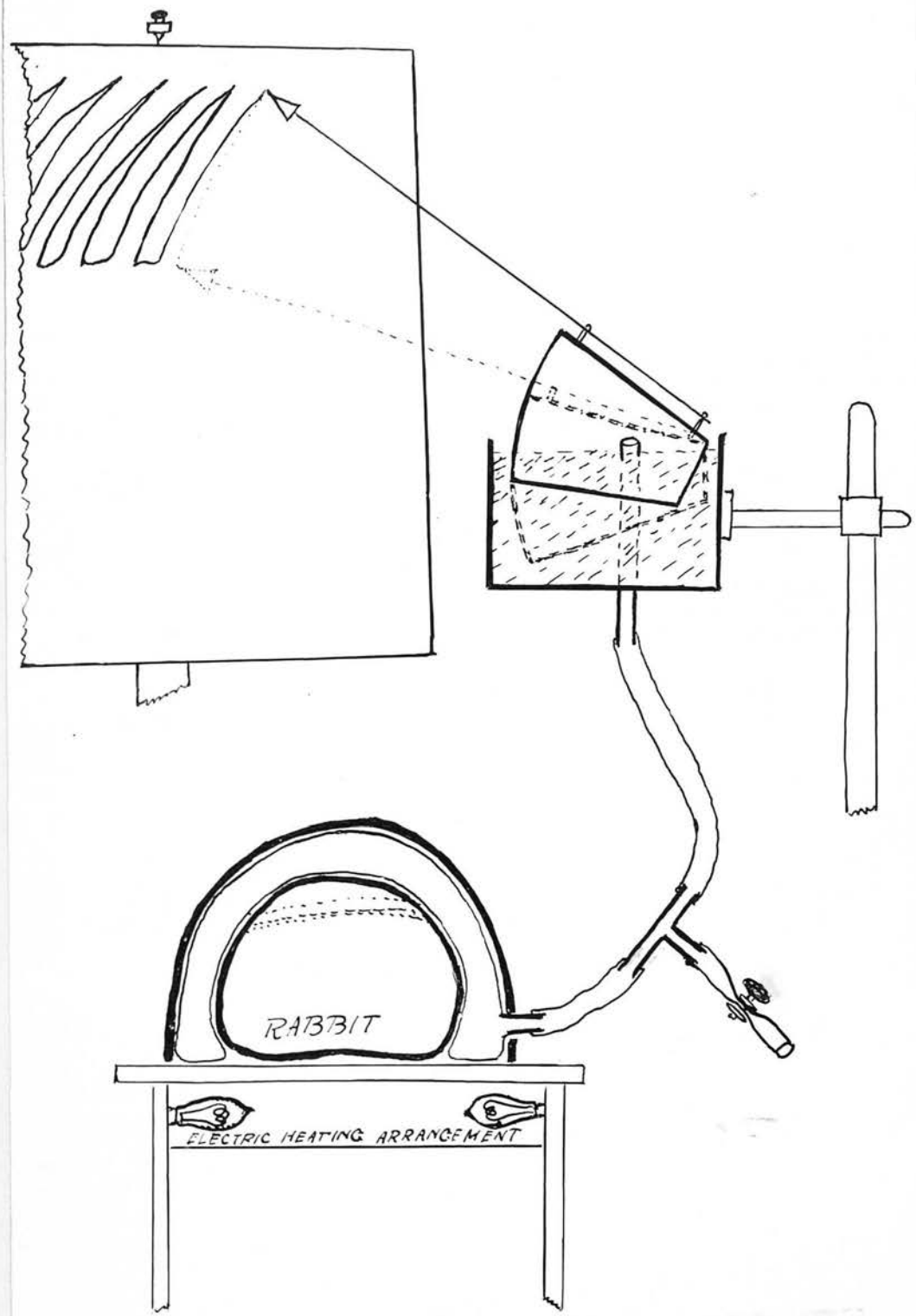


Fig. 1. Diagrammatic sketch of the float recorder and the respiratory plethysmograph. (Detailed description in the text).

experiment. For convenience of comparison, the figure for normal respiration was expressed as 100 and all other figures were converted into percentages of the normal respiration. When these percentage figures were plotted against time, a curve was obtained that graphically represented the respiratory activity of the animal for the whole period of the experiment.

Experimental Precautions. Certain precautions had to be taken to avoid possible sources of error in this method.

(1) The animal had to be handled as gently as possible and care was taken to avoid any sound or other source of nervous excitement of the rabbit - a naturally timid animal. Even when this was done, it was often found necessary to wait for ten to twenty minutes or even more until the animal's respiration became regular and normal. The amount of respiratory activity in the normal condition was, of course, a figure of great importance in the construction of the respiratory curve of the whole experiment.

(2) /

(2) Another important factor was the maintenance of the normal body temperature, as variations in temperature were noticed to cause changes in the respiratory activity of the rabbit. A rise of temperature increased respiratory activity and respiratory depression resulted from a fall of temperature below normal, to a certain extent beyond which shivering ensued.

Arrangements were therefore made to maintain steady body temperature. The top of the experimental table was made of a sheet of copper. Electric bulbs fitted below this sheet heated the sheet nearly uniformly. The animal was placed on this, with a pad of cotton beneath its body to avoid direct heat of the sheet. Frequent reading of a thermometer placed inside the rectum, was the guide to the putting off or putting on of the electric bulbs, so as to maintain a rectal temperature between 37.5°C. and 38°C.

(3). The maintenance of a uniform rate of flow of the fluid infused was very important as the whole success of the experiment depended on this. The/

The usual drop method from a burette was used at first, a constant pressure being provided by a Mariotte tube arrangement. As the size of a drop varied slightly with the rapidity of falling of a drop as well as with the size of the dropping point, the counting of the drops alone could not be relied on for a uniform rate of flow, but in addition to this, the burette reading was frequently watched in order to ensure a uniform rate of flow and adjustments of clips and taps were corrected if necessary. For the sake of accuracy, the quantity infused could not be reduced too much, and the minimum quantity of fluid that had to be used per minute with accuracy insured, caused free diuresis, washed out the drugs and thus lowered their concentration. All these difficulties were eliminated by the use of an apparatus which mechanically injected 0.1 c.c. per minute at a uniform rate.

The perfusion apparatus (Fig. 2) consisted essentially of a wheel, a long square rod and a 10 c.c. record syringe.

Fig. 2/

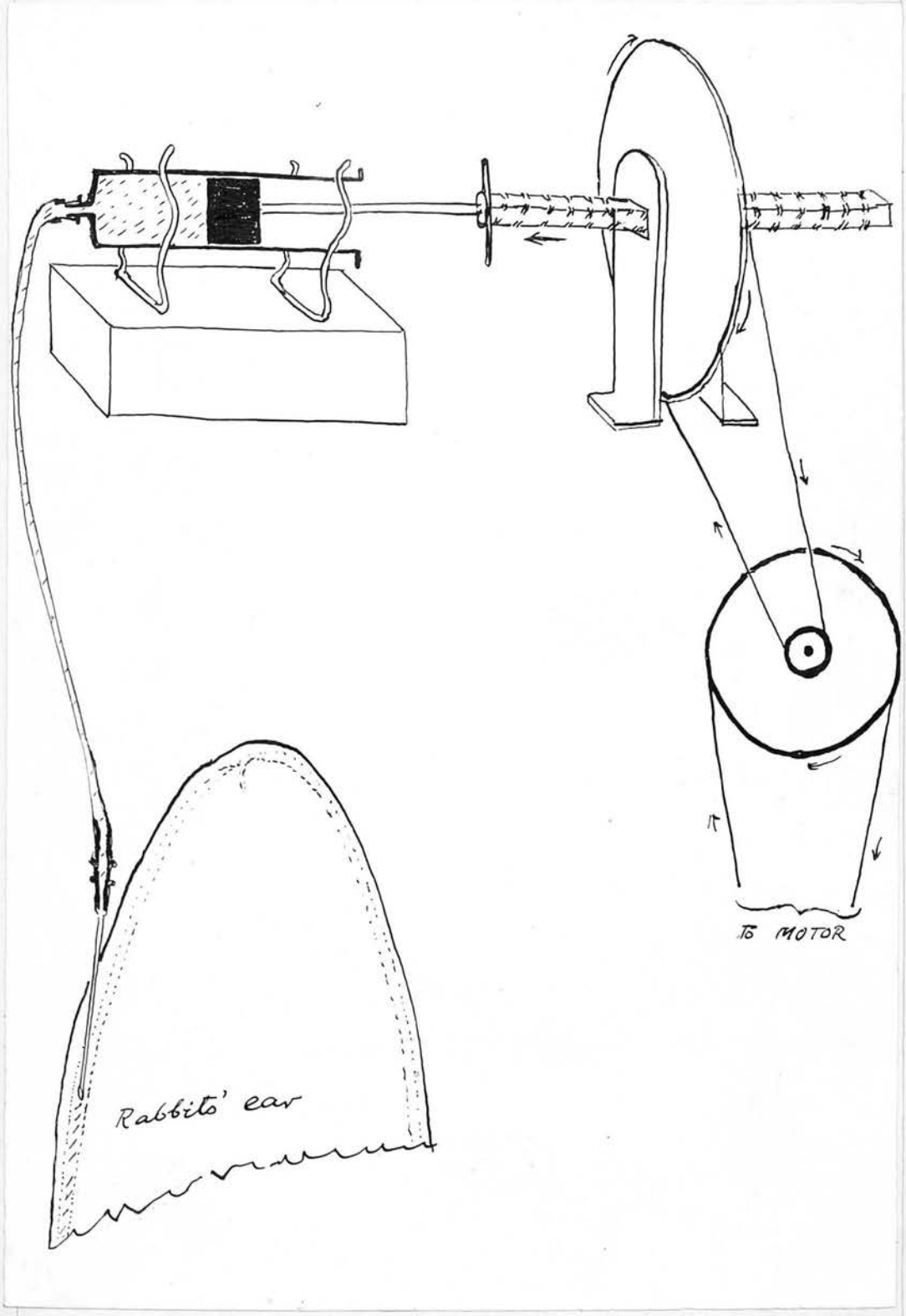


Fig. 2. Diagrammatic sketch of the continuous diffusion apparatus. (Detailed description in the text).

The wheel was placed between two supporting pillars and was rotated by means of belting from an electric motor, with the intervention of a system of reducing gears to obtain a very slow speed. The wheel had screw threads cut in a circular hole through its centre, and corresponding threads were cut at the four angles of the square rod, which passed through the circular hole of the wheel and also through two square holes of the two supporting pillars. A knob at the end of the rod touched the back of the handle of the piston of the syringe, the barrel of which was clamped to a stand. The rotation of the wheel tended to rotate the square rod, but the square holes of the supporting pillars prevented this and a forward movement of the rod was caused, equal to the distance between two screw-threads, for each complete rotation of the wheel. This resulted in the piston of the syringe being pushed forward to the same extent. The combination of gears used permitted the injection of 1 c.c. in 10 minutes, i.e. 0.1 c.c. in one minute. By altering the combinations of gears and using syringes of different bores, the rate of injection could/

could be reduced or increased ten times.

Limitations.

The method of producing a depression of the respiratory centre by evipan and its antagonism by analeptics yields satisfactory results. The quantitative accuracy of the method, however, is affected by some limitations.

(1) Individual variation. A good deal of individual variation is noticed in the amount of effect produced by the same dose (per kg.) of evipan on different animals. The accuracy of calculations about the rate of excretion of evipan on data obtained by this method is therefore subject to this factor.

(2) Recovery process. This is complicated by the fact that when the respiratory centre is depressed, CO_2 accumulates, and as the depressant drug is removed and depression relieved, the CO_2 stimulates the respiratory centre. The recovery rate is therefore quicker than would be produced by the rate of elimination of evipan. In many cases, the respiratory activity rises above normal during recovery/

recovery. Thus this factor affects the figures about the rate of elimination of evipan calculated on the time of recovery.

If these limitations are recognised, the method is very useful in studying the problems connected with narcotic analeptic antagonism.

Results.

Preliminary experiments showed that the most satisfactory method for producing a steady depression of respiration was to tranquillise the rabbit with an initial dose of 15 to 20 mg. per kg. of evipan and then to continue with continuous infusion and see whether further depression or recovery occurred.

Fig. 3 shows that respiration and blood pressure of a rabbit were both depressed after 1 mg./kg. of evipan followed by 0.6 mg./kg. per minute continuous infusion. But whereas the blood pressure soon returned almost to its former level, the respiratory activity continued to remain depressed (at 48 per cent. below normal, calculated by the above described method).

Fig. 3/

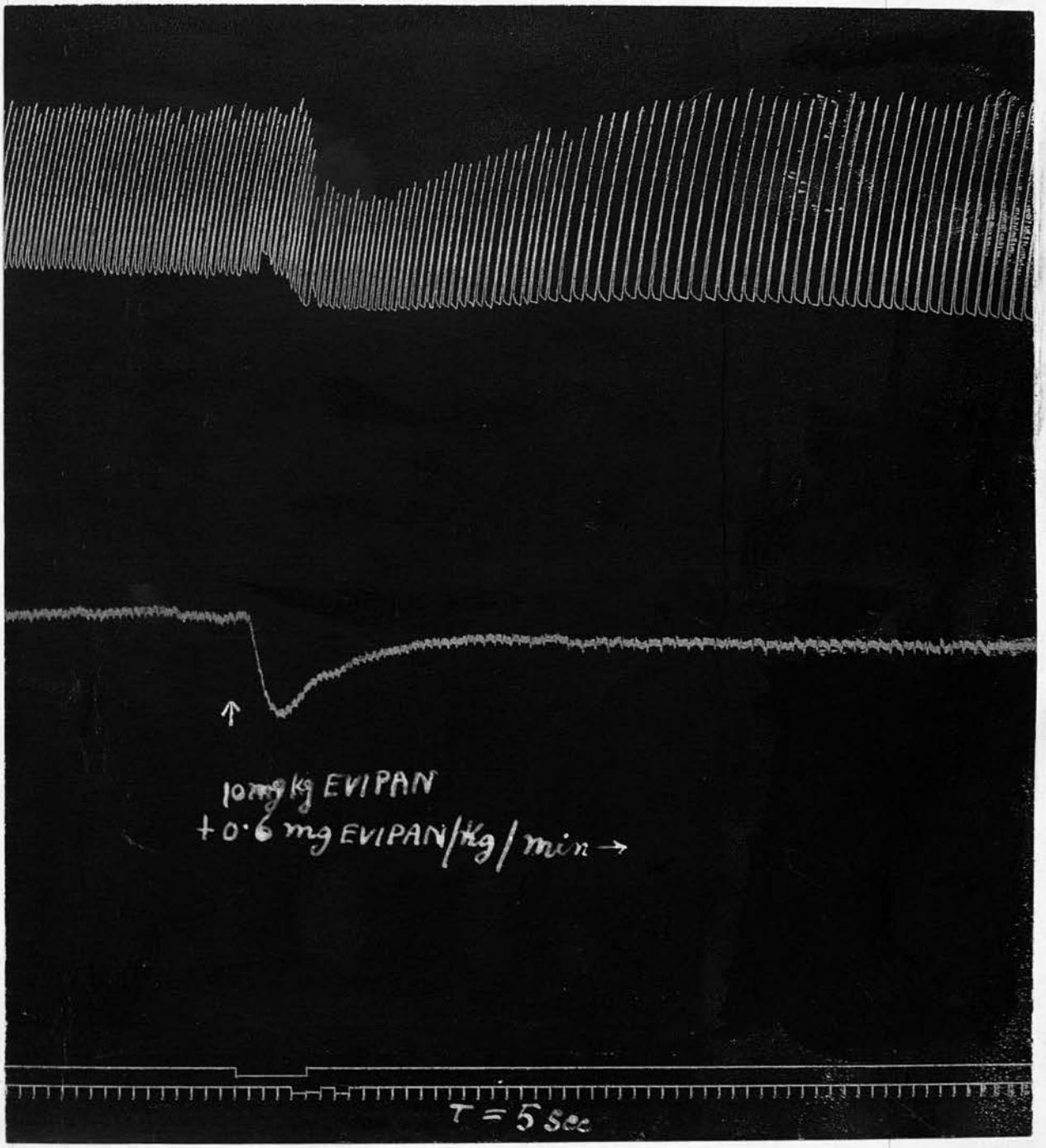


Fig. 3. Upper curve: respiration; lower curve: blood pressure. Time 5 secs. The rabbit received 10 mg./kg. of evipan followed by 0.6 mg./kg./min. continually infused. It can be seen that blood pressure recovered after some time but the respiration continued to remain depressed.

In order to estimate the probable concentration of evipan which corresponds to a given degree of respiratory depression, it was necessary to construct a curve relating evipan dosage and corresponding depression.

A dose of sodium evipan rapidly injected into the ear vein of the rabbit causes an immediate depression of the respiratory activity. The animal, however, quickly begins to recover and soon returns to normal. (In many cases, the respiratory activity rises above normal during recovery on account of CO_2 accumulation, as mentioned before). The respiratory curve, after acute evipan, shows an almost vertical fall and a rapid rise back to the normal level.

Unfortunately there is a wide individual variation in respect of sensitivity to evipan. The author has collected results from his own work and has been kindly supplied with additional data by Dr Raventos. These figures are shown in Table I and Fig. 4.

Table I /

Table I.

Respiratory depression after acute evipan.

	Mg./Kg.					
	5	10	15	20	30	
No.of experiments in ( )	(3)	(4)	(8)	(23)		Das
Percentage of respiratory depression	15	27	33	54		
No. of experiments in ( )	(7)	(9)		(9)	(5)	Raventos
Percentage of respiratory depression	43	49		50	62	

From the curve obtained in Fig. 4 the concentration of evipan necessary to produce any given depression can be read off.

The results obtained by continuous infusion of evipan are summarised in Table II and also represented by curves in Figs. 5, 6, and 7.

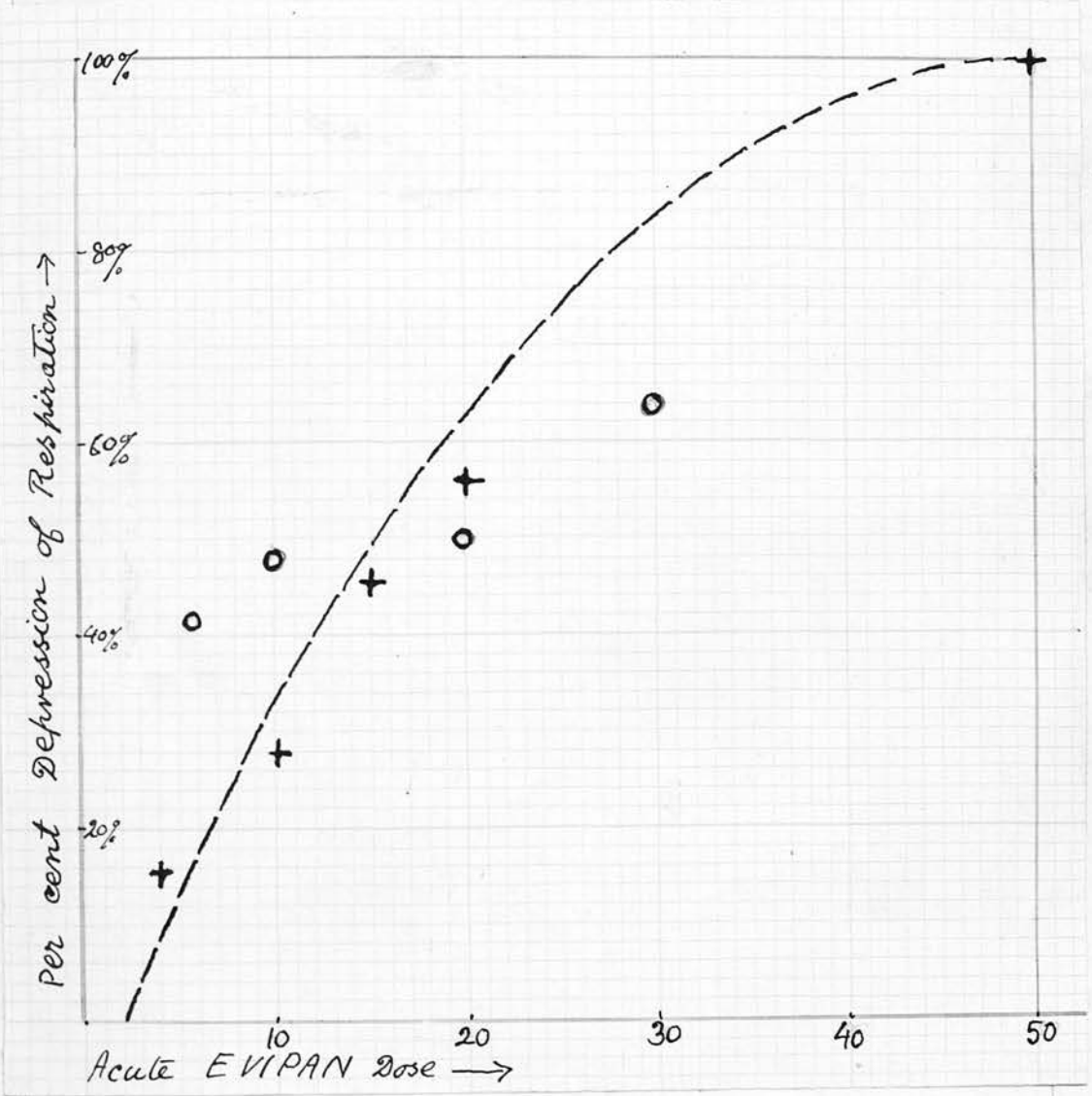


Fig. 4. Curve showing the extent of respiratory depression produced by acute intravenous injection of evipan.

\+ values obtained by author given in Table I.

0 values obtained by Dr Raventos in Table I.

Table II.

Initial dose of evipan, mg. per kg.	% of depression of resp. by initial dose.	Continuous infusion mg. per kg. per min.	Effect produced by the infusion on the depression produced by initial dose.	% of depression at which steady state reached.	No. of curve representing the expt. in the Figs. 5, 6 and 7.
20	50	0.35	Gradual recovery till steady state reached	Nearly at 25	Curve (1), Fig.5.
20	52	0.5	do.	at 34	Curve (2), do.
20	55	0.5	do.	" 36	" (3), do.
20	62	0.5	do.	" 39	" (4), do.
20	55	0.5	do.	" 41	" (5), do.
20	57	0.5	do.	" 45	" (6), do.
20	60	0.6	do.	" 48	" (7), do.
20	67	0.8	Nearly steady from beginning	" 58	Curve (1), Fig.6
20	62	0.8	do.	" 62	" (2), do.
20	68	1.0	do.	" 63	" (3), do.
20	67	1.0	do.	" 65	" (4), do.
15	22	1.5	Increase in depression		Curve (1), Fig.7
15	32	1.5	do.		" (2), do.
15/					

Table II contd.

Initial dose of evipan, mg. per kg.	% of depression of resp. by initial dose	Continuous infusion mg. per kg. per min.	Effect produced by the infusion on the depression produced by initial dose	% of depression at which steady state reached	No. of curve representing the expt. in the Figs. 5, 6 and 7.
15	25	1.5	Increase in depression		Curve (3), Fig. 7
15	35	1.5	do.		" (4), do.
10	25	1.5	do.		" (5), do.
15	42	1.5	do.		" (6), do.
15	50	1.2	do.		" (7), do.
10	42	2.0	do.		" (8), do.

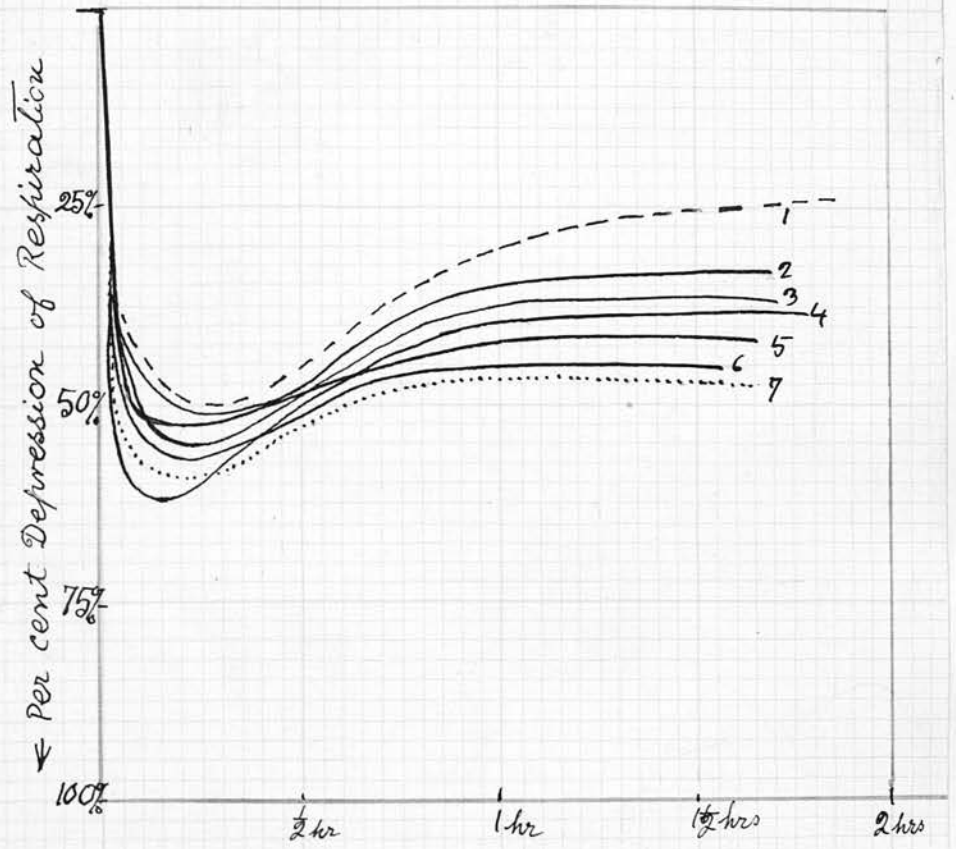


Fig. 5. Superimposed respiratory curves of the experiments summarised in top part of Table II. Initial dose was 20 mg./kg. in all cases. Continuous dose was 0.35 mg./kg./min. for curve 1 (broken line - - -) and 0.6 mg./kg./min. for curve 7 (dotted line .....) and 0.5 mg./kg./min. for all other cases.

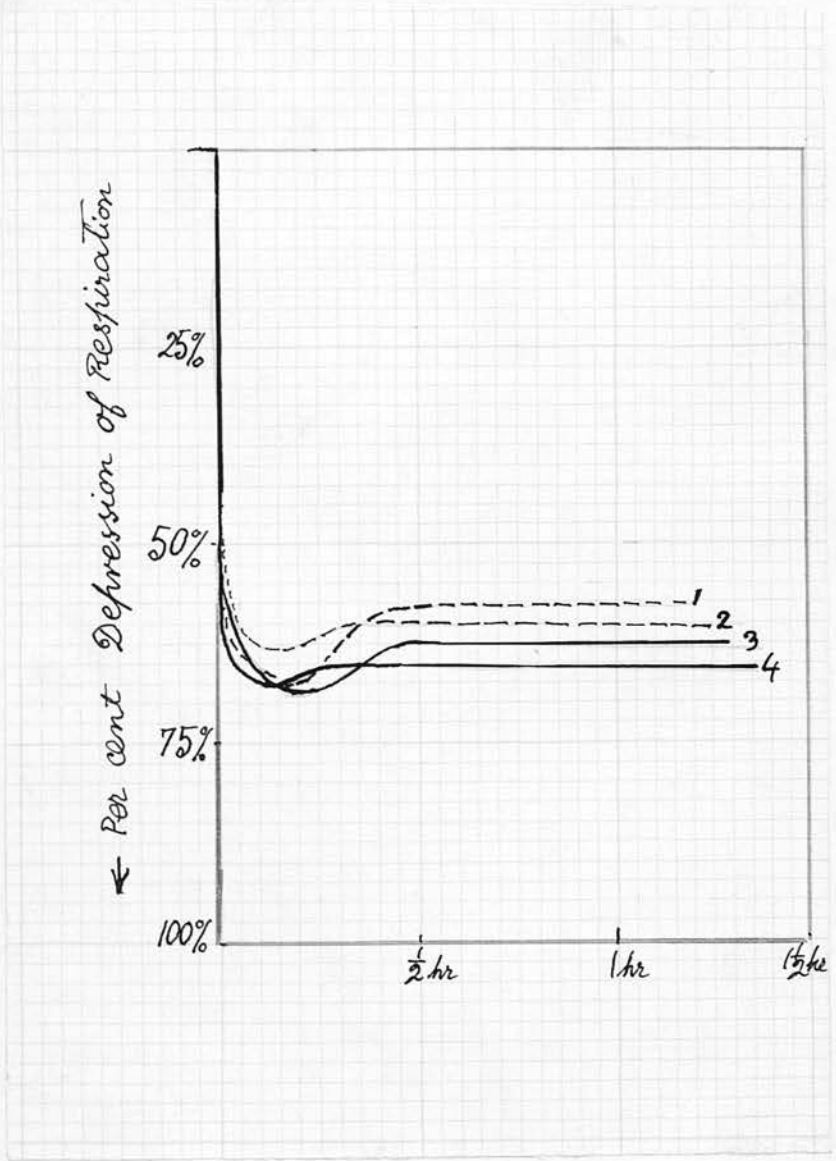


Fig. 6. Superimposed curves of experiments summarised in the middle part of Table II. Initial dose 20 mg./kg. for all cases. Continuous dose 0.8 mg./kg./min. for curves 1 and 2 (broken lines - - -) and 10 mg./kg./min. for curves 3 and 3 (continuous lines _____).

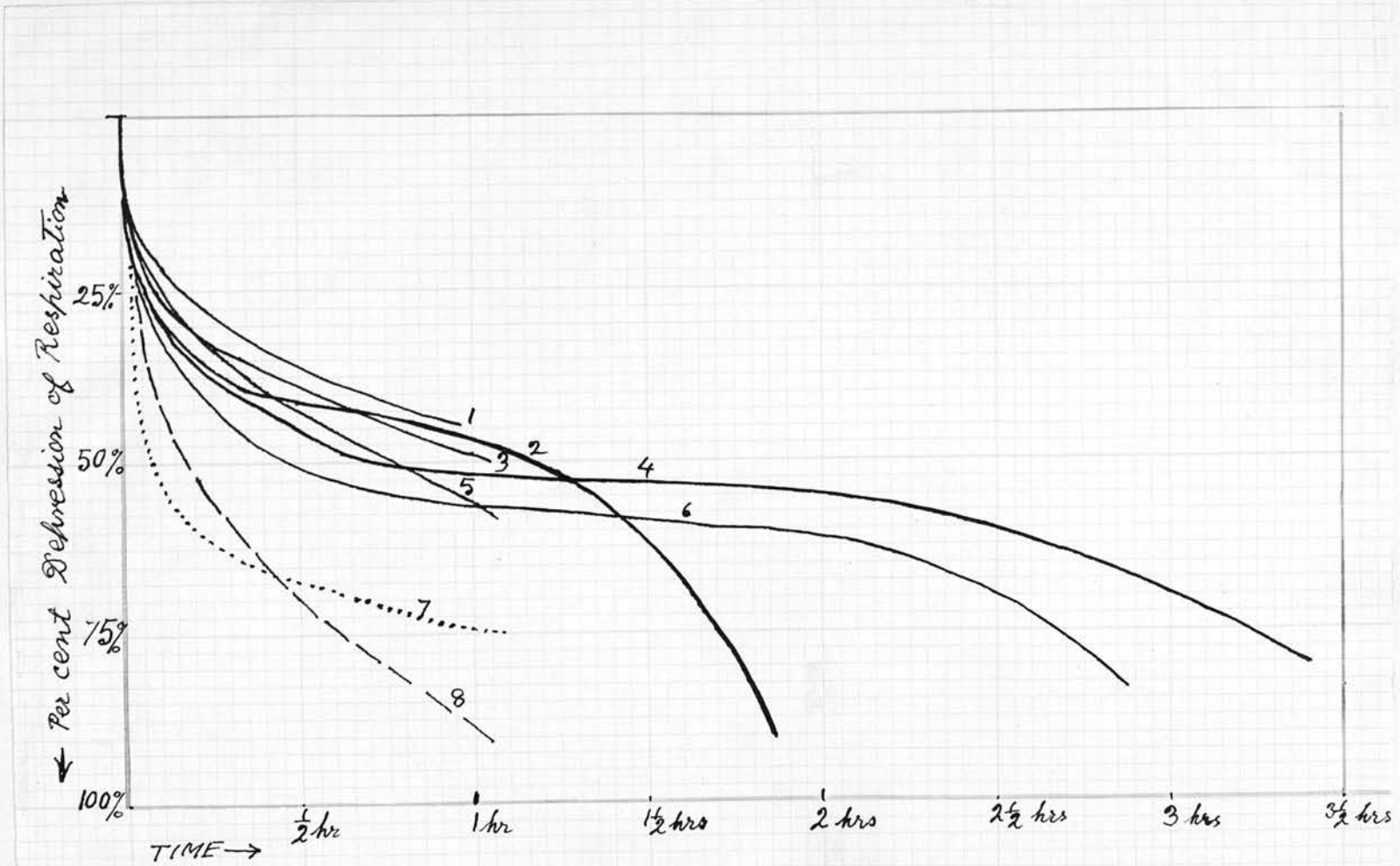


Fig. 7. Superimposed curves of experiments summarised in the bottom part of Table II. Initial dose 10 mg./kg. for curves 5 and 8 and 15 mg./kg. for all other cases. Continuous dose 1.2 mg./kg./min. for curve 7 and 2.0 mg./kg./min. for curve 8 and 1.5 mg./kg./min. for all other cases.

The average results can be described as follows:

(a) Intravenous infusion followed by recovery (cf. Fig. 5).

An injection of 20 mg./kg. of evipan produced an immediate depression of between 50 to 65 per cent. A continuous intravenous infusion of 0.5 mg./kg. per minute, following the above initial dose, resulted in slow recovery. The depression of respiration returned to about 40 per cent. in about 30 to 60 minutes and then remained steady. Presumably, at this point, the removal and introduction of the drug were equal. The amount of respiratory depression corresponds to an evipan blood concentration of 13 mg./kg. (cf. Fig. 4). and since the condition is steady with the introduction of 0.5 mg./kg./min., therefore the proportion destroyed per minute must be 0.5/13 = 1/26 of the amount present.

(b) Steady state. (cf. Fig. 6).

With an initial dose of 20 mg./kg. and a constant intravenous infusion of 0.8 to 1.0 mg./kg. the respiratory depression was constant at about

60/

60 per cent. which corresponds to an evipan concentration of 25 mg./kg. (cf. Fig. 4). This suggests that the introduction and removal were balanced from the onset. Hence the destruction must have been about 0.8 to 1.0/25 = about 1/25 of the evipan present.

(c) Further depression (cf. Fig. 7).

After an initial dose of 15 mg./kg. followed by a continuous infusion of 1.5 mg./kg. per minute respiratory depression increased fairly rapidly. In one case (curve 2, Fig. 7) respiratory arrest nearly occurred after 100 minutes, and in another (curve 4, Fig. 7) after 200 minutes. The amount of evipan needed to produce rapid respiratory arrest is between 30 to 50 mg./kg. It may therefore be concluded that an infusion of 1.5 mg./kg. per minute raised the blood evipan from 15 mg./kg. to 40 mg./kg. in about 100 minutes. If 1/25 of the evipan present were destroyed per minute, then the rate of destruction would balance an infusion of 1.5 mg./kg., when the blood concentration was 38 mg./kg. Fig. 2 indicates that probably the constant maintenance of a blood concentration of 38 mg./kg. would produce a respiratory arrest.

The/

The following experiments were made to provide as conclusive a proof as possible of the nature of the clearance of sodium evipan. An experiment of the type shown in Table II was made on a rabbit. A large dose (50 mg./kg.) of sodium evipan was injected cautiously so that respiratory depression just short of arrest was produced. This was followed by continuous infusion of 1 mg./kg. minute of sodium evipan. With this treatment the animal recovered to a steady state of 30 per cent. depression. This effect can only be accounted for by the assumption that a fixed proportion of the drug present is cleared per minute. This conclusion was confirmed by making another experiment on the same rabbit (to eliminate individual variation) a few days later. In this case no initial dose of evipan was given, but only a continuous injection of 1 mg./kg./min. This caused a slow fall of respiratory activity to the same steady state that was finally reached in the previous experiment. Three pairs of such experiments were made with similar results. Fig. 8 shows one pair of these experiments.

Fig. 8/

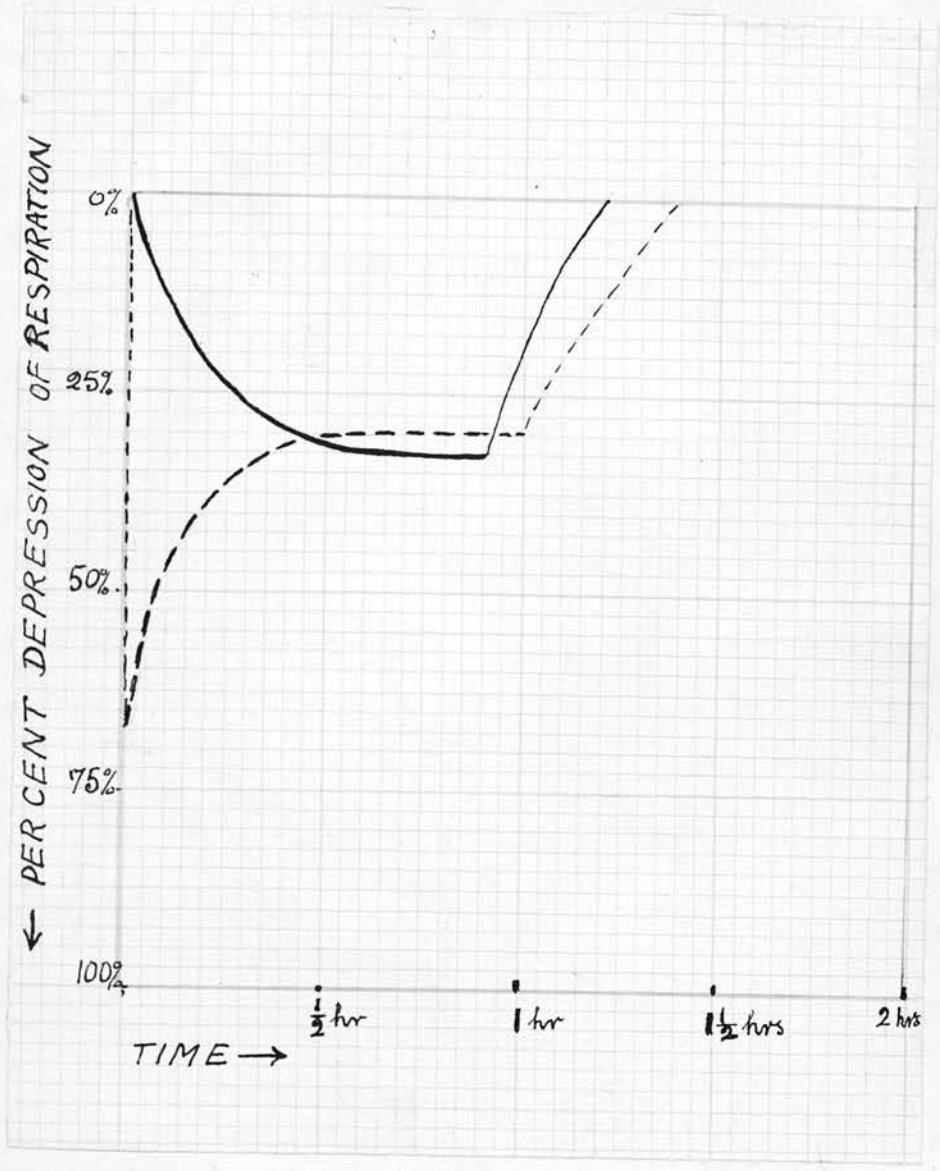


Fig. 8. Broken line (- - -) = the respiratory curve of a rabbit that received 50 mg./kg. of evipan slowly given i.v. followed by 1 mg./kg./min. continuous infusion. Continuous line (_____) curve of the same animal receiving 1 mg./kg./min. of evipan without any initial dose, 4 days later. Both attained steady course at nearly same level.

Since the continuous infusion was the same in both cases, the approximation to the same final steady state cannot be explained by removal of a constant quantity of the drug per minute, whereas this result is to be expected if a constant proportion of the drug present is removed per minute.

In another experiment (of the type of the second part of the above pairs of experiments), a continuous injection of 1 mg./kg./min. without any initial dose, was maintained for 8 hours. The respiratory depression, after the slow fall in the beginning, assumed a steady state which was thereafter maintained throughout the whole period of this lengthy experiment. (See Fig. 9)

These experiments therefore provide fairly conclusive proof that the proportion and not the amount of drug removed per minute is constant.

Discussion.

(a) These experiments with prolonged perfusion demonstrate certain facts of interest. An acute injection of less than 50 mg./kg. of sodium evipan is sufficient to arrest respiration in the rabbit. In one experiment (curve 4, Fig. 7), 1.5 mg./kg./min. was/

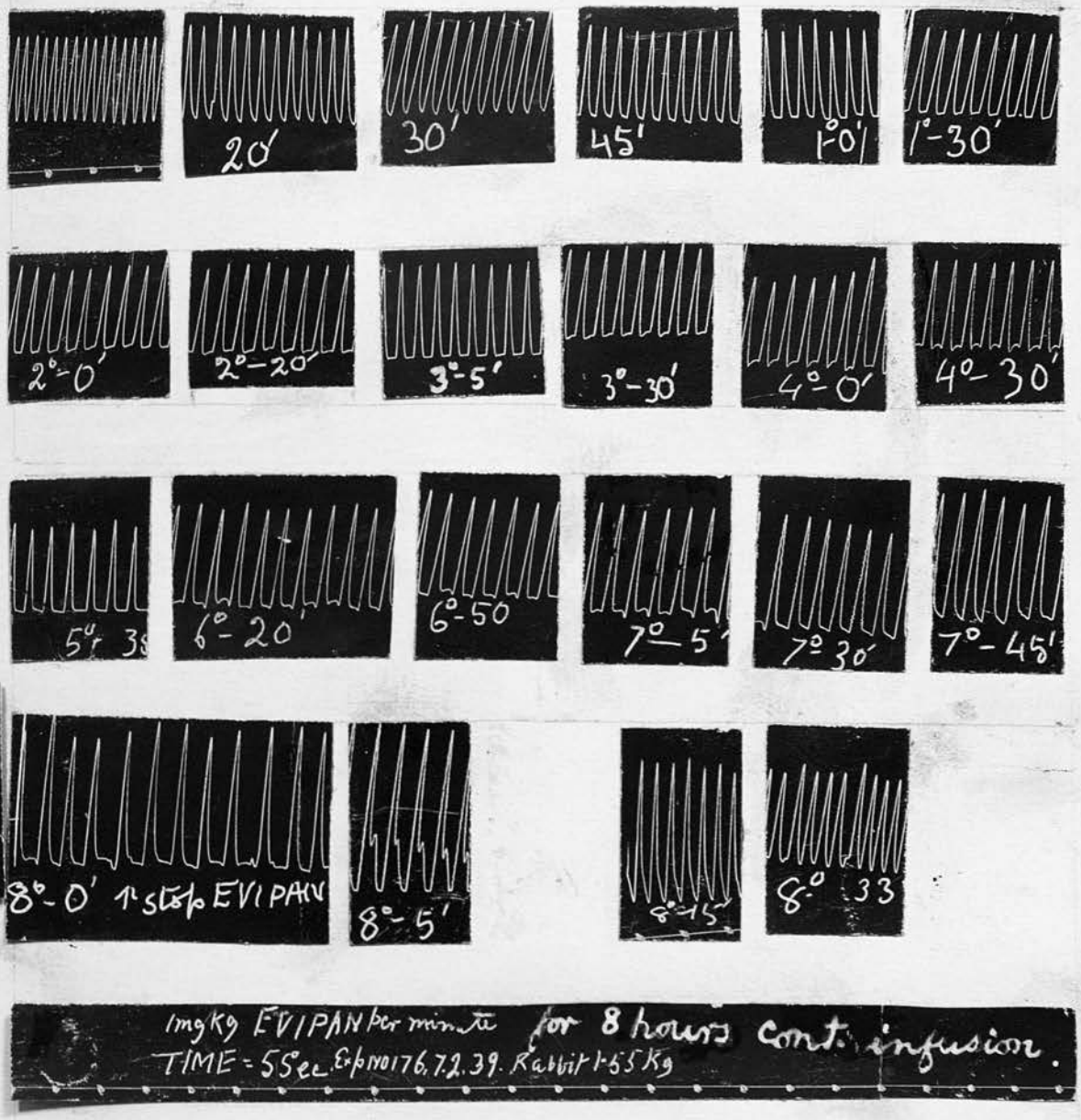


Fig. 9. Portions of respiratory tracing of a rabbit that received a continuous infusion of 1 mg./kg./min. of evipan (without an initial dose) for 8 hours. Respiratory depression became steady in about an hour and continued so for 7 hours.

was infused for 180 minutes after an initial dose of 15 mg./kg. Hence the rabbit received a total of 285 mg./kg. of sodium evipan. This is nearly six times the acute lethal dose. It is improbable that such a quantity could be detoxicated by fixation by tissues such as subcutaneous fat. Hence such results provide fairly certain evidence of the breakdown of evipan.

(b) There are two methods of breakdown of drugs.

(i) In the case of alcohol (and of substrates destroyed by enzymes when the substrate is present in excess), a fixed amount is oxidised per minute and this amount is not affected by the concentration In these cases there can be only one rate of continuous infusion which can produce equilibrium, namely, that which exactly equals the rate at which the body can destroy the drug.

(ii) In the case of most drugs (and of substrates destroyed by enzymes when the substrate is not in excess), a fixed proportion of the drug present is broken down per minute (e.g. acetylcholine, Clark and Raventos, 1938). With this mode/

mode of destruction, continuous infusion can produce equilibrium at different levels.

The results described above which were observed with continuous infusion of evipan, can only be explained on the second hypothesis. If, for example, the body always removed 1.0 mg./kg. per minute of evipan, then this would explain the steady depression produced by this dose, but with an initial dose of 20 mg./kg., an infusion of 0.5 mg./kg. per minute and a constant destruction of 1.0 mg./kg. per minute, almost complete recovery would occur in 40 minutes. Similarly with an initial dose of 15 mg./kg., a constant infusion of 1.5 mg./kg. per minute and a destruction of 1.0 mg./kg. per minute, a lethal quantity (about 45 mg./kg.) would accumulate in 60 minutes.

On the other hand, the results can be interpreted satisfactorily on the hypothesis that a constant proportion is destroyed. Figs. 5 and 6 show that equilibrium can be attained at more than one rate of continuous infusion. With a continuous infusion of 0.5 mg./kg. per minute the level of depression is equal to that produced by about 13 mg./kg. With a constant infusion of 1.0 mg./kg. the/

the level of depression is equal to that of 20-25 mg./kg. Clark and Raventos (1938) calculated the cumulation produced by intermittent administration of drugs, when a constant fraction of the amount present is broken down per unit of time. These calculations apply approximately to continuous infusion and it may be assumed that if a drug is introduced at X units per minute and a constant fraction (1/y) of the amount present is destroyed per minute, then the concentration (C) ultimately produced will be xy(C = xy). As 0.5 and 1.0 mg./kg. constantly infused produce steady depression of 40 per cent. and 60-65 per cent respectively, Fig. 4 provides the following two equations:-

$13 = 0.5\ y$ and $25 = 1.0\ y$

This gives $y = 25$.

If 1/25 of the amount present is destroyed per minute, then half destruction occurs in 19 minutes. This result is in accordance with observations on the duration of action of evipan.

Dr Raventos has kindly permitted me to quote the following figures for the relation between/

between the dosage of evipan and the duration of depression of respiration. These were obtained with a single rabbit. The individual variation is so wide that it is difficult to compare results obtained with different animals.

Table III.

Dose of sodium evipan in mg./kg.	10	15	20	25	35
Duration of depression of respiration in minutes	10	20	25	40	50

These times can be interpreted on the assumption that the minimum effective dose is 7 mg./kg. and that half destruction occurs in 20 minutes.

According to the calculations made above, a continuous infusion of 1.5 mg./kg./min. of evipan would produce a concentration equal to 25 x 1.5 or 38 mg./kg. This is a dose which frequently causes/

causes respiratory arrest and hence the steady increase of respiratory depression obtained with this rate of infusion agrees with the hypothesis outlined above.

(c) Continuous intravenous infusion of a drug with a mode of destruction like that of alcohol soon leads to cumulation of greater and greater amounts. But no cumulation occurs after equilibrium is established in the case of drugs whose definite fraction is destroyed per unit of time. The information that a definite fraction of evipan is destroyed per unit of time therefore is of practical importance in its use as an intravenous anaesthetic by constant infusion of a suitable dose, which can be continued for prolonged periods with the confidence that no further accumulation would occur beyond the equilibrium level.

Summary/

Summary.

These experiments with evipan prove the following facts:-

1. The method of estimating respiratory activity that has been described, provides a convenient quantitative measure.

2. Continuous infusion of sodium evipan will produce a respiratory depression that remains constant in its intensity for hours.

3. Steady respiratory depression at different levels can be produced by varying the rate of intravenous infusion of sodium evipan.

4. The results can be interpreted on the assumption that a constant fraction of the evipan present in the body is destroyed per minute. The results indicate that about 1/25 of the evipan present is destroyed per minute. This equals half destruction in about 20 minutes.

5. This information about the mode of destruction of/

of evipan renders it suitable for use as an intravenous anaesthetic by continuous infusion method, with the confidence that the margin of safety will not be encroached upon beyond the equilibrium level.

IV. MEASUREMENT OF ANALEPTIC EFFICIENCY.

The problem of the analeptic activity of drugs has been approached by workers in different ways. In the first place some authors have studied hypnotic and analeptic activity on isolated organs and tissues. Jowell and Quastel (1937) studied the oxygen consumption of brain slices under varying conditions. Porter and Allamon (1936) avoided the use of whole animals as being "subject to vagaries of the intact nervous system and difficulty of finding reliable criteria for measuring depth of anaesthesia" and used spinal cats. With the help of special types of "liquid electrodes", they studied the threshold of the flexian reflex and found an increase of the threshold under barbiturates and a lowering thereof after strychnine. The results of this interesting work, useful so far as the isolated spinal cord is concerned, cannot unfortunately be taken as a real standard of the analeptic efficiency of strychnine against the barbiturates in the intact cat. In fact, it is these "vagaries" that count in the matter of the analeptic activity and the true evidence of the analeptic activity has to be obtained from intact animals with no (or as little as/

as possible) interference with their anatomical and physiological condition. Most workers have therefore employed intact animals.

Nielson, Higgins and Spruth (1925), Eddy (1928) and Shonle, Keltch and Swanson (1930) have used the diminution in the depth of barbiturate depression (judged by subjective methods) produced by analeptics as the ratio of their efficiency. Personal errors in such subjective methods must necessarily be large enough to render the results inaccurate.

Anderson, Chen and Leake (1930) used the diminution of basal metabolism as a measure of the degree of depression. Kranz, Car and Beck (1937) determined the oxygen consumption of white rats, in normal conditions, after nembutal, and after nembutal followed by picrotoxin, and showed from average figures that picrotoxin restored the oxygen consumption to normal when this had been depressed by nembutal.

This method has the disadvantage that there is a good deal of variation in the individual response of different animals and there are drugs like adrenaline that increase the oxygen consumption of normal animals as well as narcotised ones without antagonising/

antagonising the narcotics.

Barlow (1930) determined the concentration of N_2O necessary to produce anaesthesia in rats before and after analeptics. Apart from other considerations, the individual factor cannot be eliminated in this method.

Attempts have for a long time been made to estimate the analeptic value of a drug by noting the maximum amount of a narcotic against which an analeptic can protect an animal. Brown as early as 1875 showed that picrotoxin could protect rabbits against 5 to 8 times the lethal dose of chloral hydrate. This method, although it gives valuable information about the efficiency of a potent analeptic, gives no definite idea as to how it behaves at different periods of its action. Similar remarks may be made about methods adopted by Maloney et al. (1931, 1933, 1935), Barlow (1935) and Koppanyi et al (1936) who noted the awakening from or shortening of drug-sleep or its prevention by different analeptics for estimating their relative efficiency.

Attempts were also made to determine the corresponding/

corresponding counterbalancing doses of hypnotics and analeptics by noting the increase in the minimum convulsive doses of analeptics caused by the previous administration of different doses of hypnotics. But the appearance of convulsions in a depressed animal is not always an indication of impending recovery, because in a deeply narcotised animal, convulsions may be produced by a stimulant drug without causing awakening, and some barbituric acid derivatives can produce hypnosis and convulsions at the same time.

Barlow stressed the importance of judging the degree of the improvement that different analeptics could produce in the respiration, the circulation and the reflex excitability of animals in barbiturate narcosis, although he relied on a subjective observation of these, without adopting any objective method of recording them.

Continental workers [(Uhlmann (1924), Helaers (1929), Hildebrandt (1926, Rein (1933)] used records of the minute volume of respiration by tracheal intubation for demonstrating the analeptic efficiency of coramine and cardiazol. But the anaesthesia required for the operation of tracheotomy deprived/

deprived them of the figure for normal conditions for comparison, and the operation did not leave the animal quite intact. Long ago Cushny (1913) discarded tracheal intubation for recording respiration volume as he observed that rabbits whose respiration was recorded by tracheal cannula, suffered from dyspnoea and further noted that the attachment of a Chauveau valve close to the trachea deepened respiratory movement.

Marshall and co-workers (1937) reported that they had some dogs trained to remain quiet on the experimental table with airtight masks fitted on to the face, and measured the inspiratory and expiratory volumes with Bohr gasometers. They attempted to revive the respiration by analeptics after causing respiratory failure by denervation of the carotid sinus or by oxygen administration in a deep condition of narcosis. (They held that this latter procedure diminished the CO_2 concentration of the blood and deprived the carotid sinus and aortic receptors of the only source of stimulus necessary for the reflex stimulation of the respiratory centre). The limitations and complications of this method weigh too much against

any/

any possible advantages it may possess. Smyth (1937) used a less complicated method for studying the effects of denervation of the carotid sinus on respiration. He enclosed the rabbit in an air-tight chamber and recorded amplitude of respiration by a float volume recorder connected with the chamber. A mask was fitted airtight over the animal's face. A tube passing airtight through the wall of the chamber established communication between the face-mask and outside and served as passage for breathing in and out of the mask. Apart from other difficulties from the position of the animal with the mask inside a small airtight chamber, the animal's body was not accessible for injection of drugs during the experiment without undoing the airtight arrangement.

That depression of respiration was the deciding factor in extremely depressed conditions, brought on by narcotics, was demonstrated by the following experiment. A steady depression was produced in a rabbit by the continuous injection of 0.8 mg./kg./min. of evipan and its blood pressure and respiration were recorded. (Fig. 10)

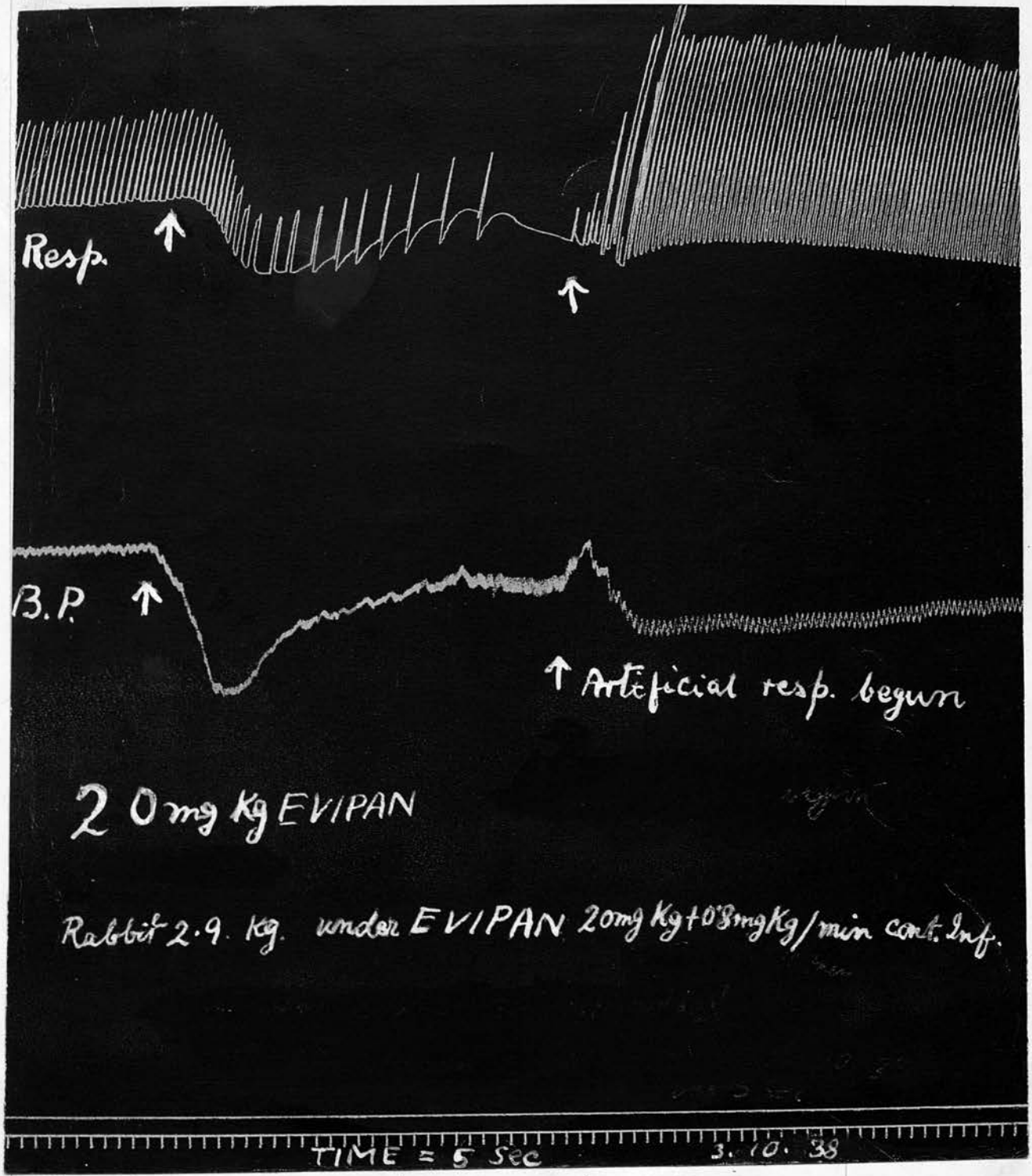


Fig. 10. Respiration (upper curve) and blood pressure (lower curve) of a rabbit under continuous evipan anaesthesia with 0.8 mg./kg./min. following 20 mg./kg. initial dose. Rapid i.v. injection of another 20 mg./kg. of evipan caused respiratory paralysis, but blood pressure after an initial fall, recovered. Animal saved by artificial respiration.

Another dose of 20 mg./kg. of evipan rapidly injected intravenously produced such a depression of the respiratory centre that respiration stopped after a few respiratory movements, while the blood pressure, after an initial fall, almost recovered its previous level, and the animal was saved from immediate death by artificial respiration.

It was found in the preliminary group of experiments that the maximum dose of continuous evipan that could be given to rabbits with safety was 1 mg./kg./min. Larger quantities produced a gradually increasing depression and caused death. Such lethal doses of continuous evipan were used in this group of experiments to determine the antagonising capacity of analeptics. By suitable adjustments, a dose of the analeptic could be found which prevented the downward slope of the curve (due to the lethal dose of evipan) and maintained it at a horizontal level during the period of continuous infusion of both the drugs (extending over $1\frac{1}{2}$ - 2 hours). This dose of the analeptic therefore antagonised the dose of the evipan given by continuous infusion. The doses of picrotoxin required

to/

to antagonise different doses of evipan were determined in this manner and it was found that the doses of evipan varied as the logarithms of the doses of picrotoxin.

The action of analeptics on the rabbit's respiratory centre when this is depressed by continuous infusion with sodium evipan, can be measured in various ways, as will be seen in the following description of the different methods employed in a study of the analeptics.

I. The simplest method was to give a single intravenous dose of an analeptic and measure the intensity and duration of its stimulant action on the respiration of a rabbit receiving a continuous infusion of evipan. The resulting increase in the respiratory activity could be read off from the respiratory curve prepared in the way described in a previous chapter. The intensity of action was indicated by the rise of the curve above the horizontal level of the curve maintained by evipan, and the duration, by the time taken by the curve to return to the horizontal level. Picrotoxin, coramine, cardiazol, strychnine/

strychnine and a number of new synthetic compounds were in this way investigated in order to determine the analeptic efficiency of each at different levels of depression. (The results of these studies will follow).

II. Comparison of different analeptics.

This method afforded special facilities for comparing the analeptic efficiency of different drugs under almost identical conditions. The analeptics produced a transient action and hence when the infusion rate of evipan was kept constant, the respiration in course of time returned to the level of depression that had been present before the administration of the analeptics. Hence it was possible to administer different analeptics alternately to the same animal and thus compare their efficiency. In this manner errors due to individual variation could be eliminated.

III. Continuous infusion of analeptics and of evipan simultaneously.

(a) Single injections of an analeptic did not give any exact idea of the rate of elimination of the drug. In other experiments, therefore, analeptics/

analeptics were given by slow continuous infusion, along with a dose of continuous evipan, which alone was sufficient to maintain a steady level of depression. Starting from a very small dose per minute, of the analeptic, and gradually increasing the dose in successive experiments, a point could be reached beyond which the respiratory curve did not maintain a steady level but showed a gradual sloping rise. Beyond this point the rate of destruction of the analeptic infused was greater than the rate of elimination and this provided an estimate of the rate of elimination of the drug at the level of the depression produced by the dose of continuous evipan administered simultaneously.

In the case of some drugs (e.g. coramine) it was noticed that the gradual rise in the respiratory curve in experiments of this group could not be maintained long, but the curve gradually came down and dropped below the level of depression due to evipan alone, in spite of the continuous infusion of the analeptic, and on stopping the infusion of the analeptic, the respiratory curve returned to the level of evipan depression. This clearly indicated a secondary depressant effect of the analeptic - a feature/

feature that would not have been demonstrated otherwise. This method also led to the detection of the limitations of drugs like strychnine in the appearance of convulsions when given by a continuous infusion long before the depression of evipan was fully overcome.

Summary.

Quantitative estimates of the activity of analeptics were obtained from the increase in the respiratory activity of rabbits whose respiration was maintained in a steady state of depression by a continuous intravenous infusion of sodium evipan (0.8 mg. to 1.0 per kg. per minute following an initial dose of 20 mg./kg.).

The intensity of action of single doses of analeptics was indicated by the rise of the respiratory curve above the steady level maintained by evipan and the duration by the time taken by the curve to get back to the previous steady level.

Different analeptics were compared under identical conditions, injecting them alternately. By simultaneously infusing both evipan and analeptics, quantitative estimations of the antagonism between the/

the barbiturate and the analeptic were obtained. This procedure also elicited valuable information about the rate of detoxication of the analeptic and evidence of secondary depressant effects, if any, possessed by the analeptic.

V. ANTAGONISM OF EVIPAN BY PICROTOXIN.

The antagonism between barbiturates and picrotoxin has been studied by various authors, and the following quantitative estimates are of interest.

Maloney and co-workers (1931) gave fixed doses of barbiturates to rabbits and followed this by varying doses of picrotoxin and thus determined the amount of picrotoxin which would cause survival. They obtained the following results.

Table IV.

Mg.	60-	80-	100-	125-	150-	175-	200
Amytal			100		150		200
Picrotoxin			3.6		4.1		20.7
Ipral					150	175	200
Picrotoxin					5.7	9.0	14.5
Nembutal	60	80	100	125	150		
Picrotoxin	2.2	2.5	4.4	12.3	32.2		
Pernocton			100	125	150		200
Picrotoxin			2.38	2.7	2.8		9.9
Phenobarbitone					150	175	200
Picrotoxin					4.3	7.3	13

Koppanyi et al. (1936) observed the awakening effects of picrotoxin in nembutal hypnosis of rabbits and gave the following figures.

Table V.

Nembutal	20 mg.	40 mg.	60 mg.	80 mg.
Picrotoxin	4 mg.	8 mg.	16 mg.	32 mg.

Fig. 11 shows the curves obtained by plotting the above figures. In practically all these cases the curves are converted into straight lines if log. doses of picrotoxin are substituted for picrotoxin doses (Fig. 12).

Figs. 11 and 12 /

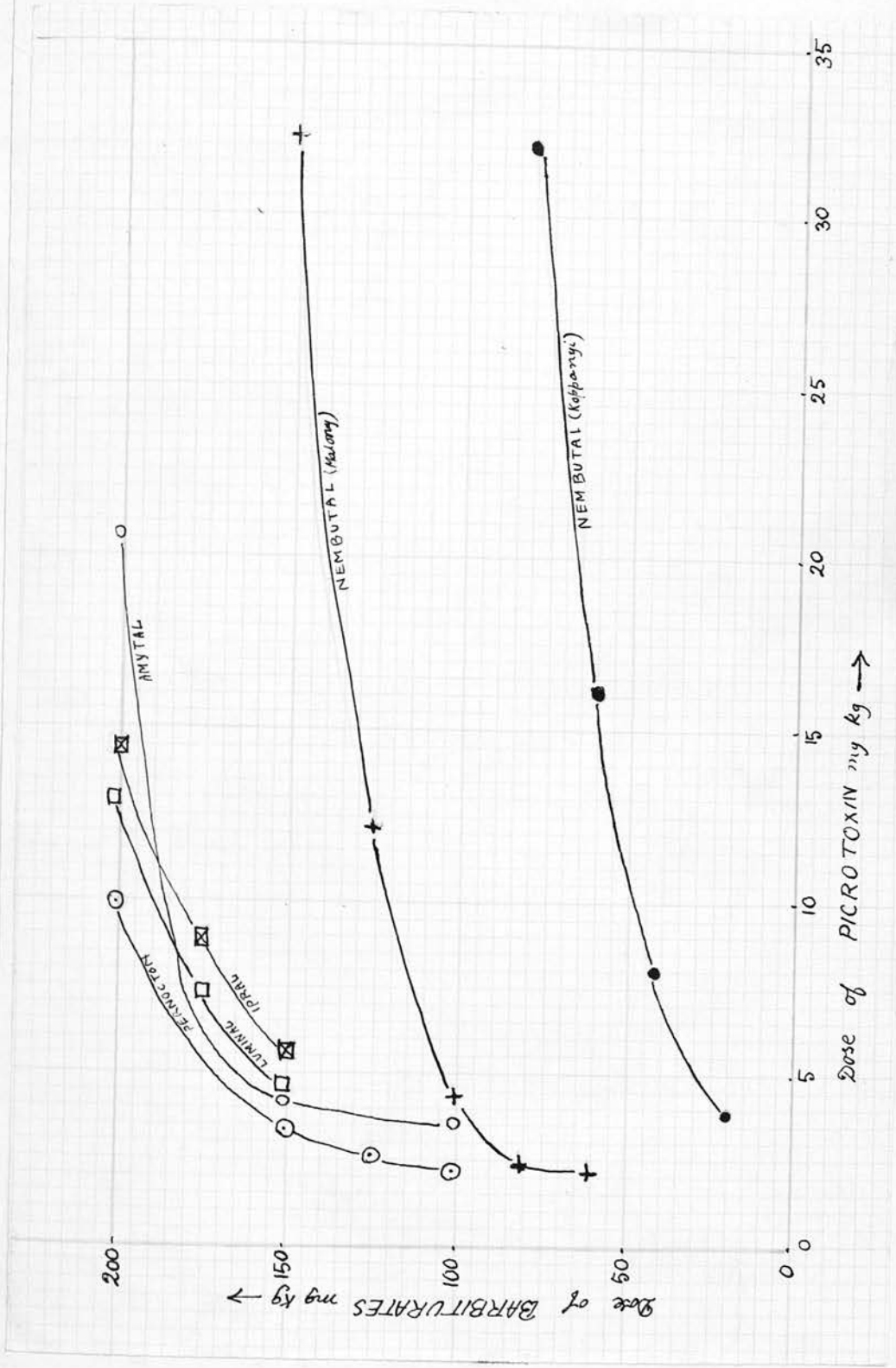


Fig. 11. Curves obtained by plotting the antagonising doses of barbiturates and picrotoxin given in Tables IV and V.

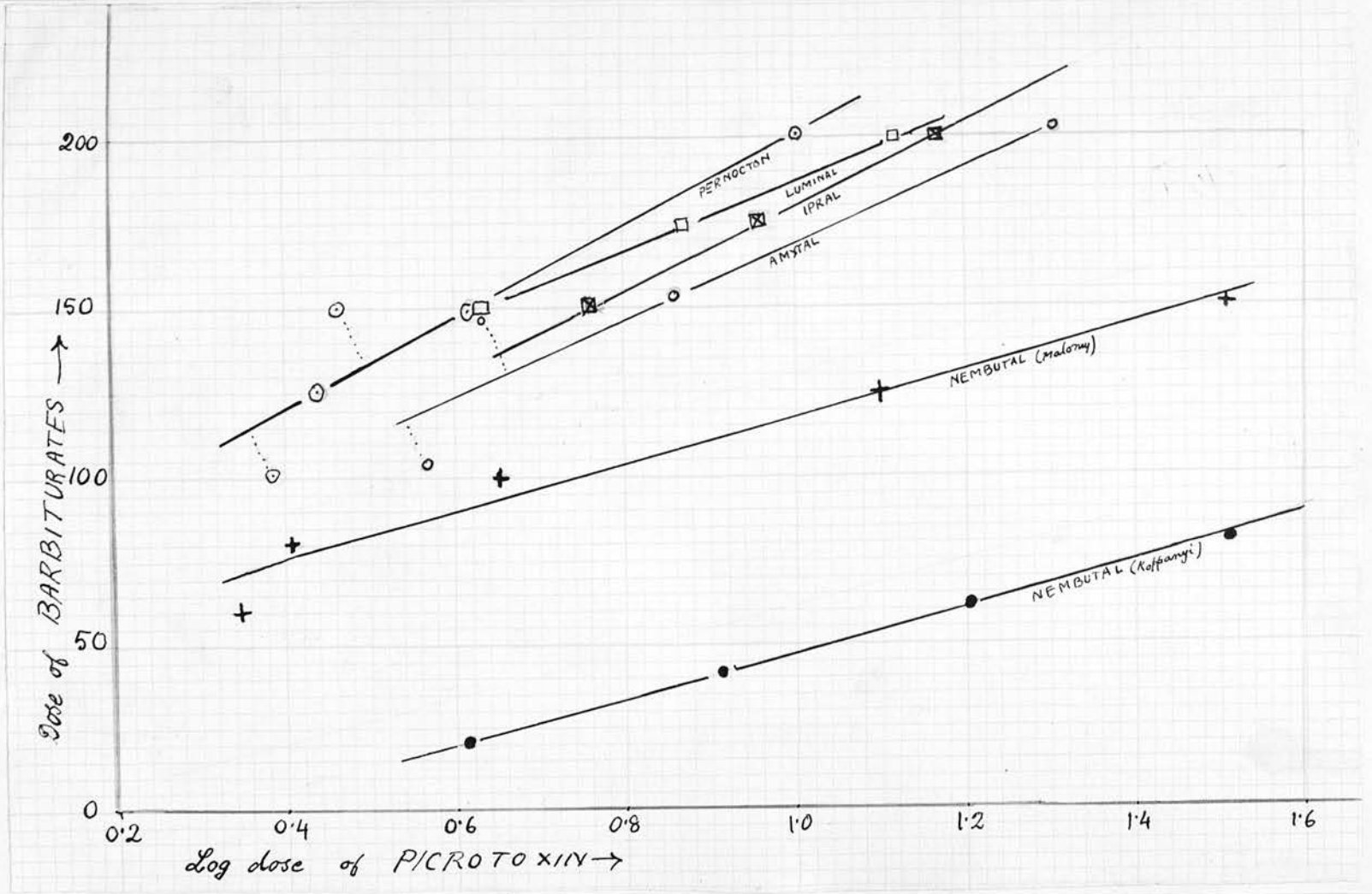


Fig. 12. Curves obtained by plotting the log. doses of picrotoxin with the doses of the barbiturates in Tables IV and V.

These curves relating dosage of barbiturate with log. dosage of picrotoxin are all nearly parallel which is surprising, because the duration of action of phenobarbitone is so much greater than that of nembutal. The general relation can be stated that it is necessary to increase the picrotoxin dosage fourfold in order to antagonise an increment of 50 mg./kg. of barbiturate. This relation explains why it is possible to antagonise such a wide range of picrotoxin dosage by barbiturates, whilst only a comparatively narrow range of barbiturate dosage can be antagonised by picrotoxin.

Experimental.

Two types of experiments were carried out. (i) Continuous intravenous evipan was given at a rate which produced a steady respiratory depression. Single doses of picrotoxin were injected and the amplitude and duration of the respiratory stimulation were measured.

The same method was employed to compare the action of picrotoxin with those of other analeptics.

(ii)/

(ii) Measurements were made of the antagonism between a continuous infusion of evipan and a continuous infusion of picrotoxin. This method permitted the balancing of evipan and picrotoxin and it was possible to work up to doses of both drugs, either of which alone would have been fatal.

(i) Antagonism of evipan by a single dose of picrotoxin.

Fig. 13 shows an example of this type of experiment. An initial dose of 20 mg./kg. followed by a continuous infusion of 0.6 mg./kg. sodium evipan produced a steady respiratory depression at 55 per cent. below normal. The injection of picrotoxin (0.3 mg./kg.) produced a temporary stimulation of respiration which lasted 20-30 minutes. The temporary nature of the effect indicates that the picrotoxin is fairly rapidly detoxicated in the body. A dose of 0.2 mg./kg. produced no certain stimulation of respiration.

Fig. 13/

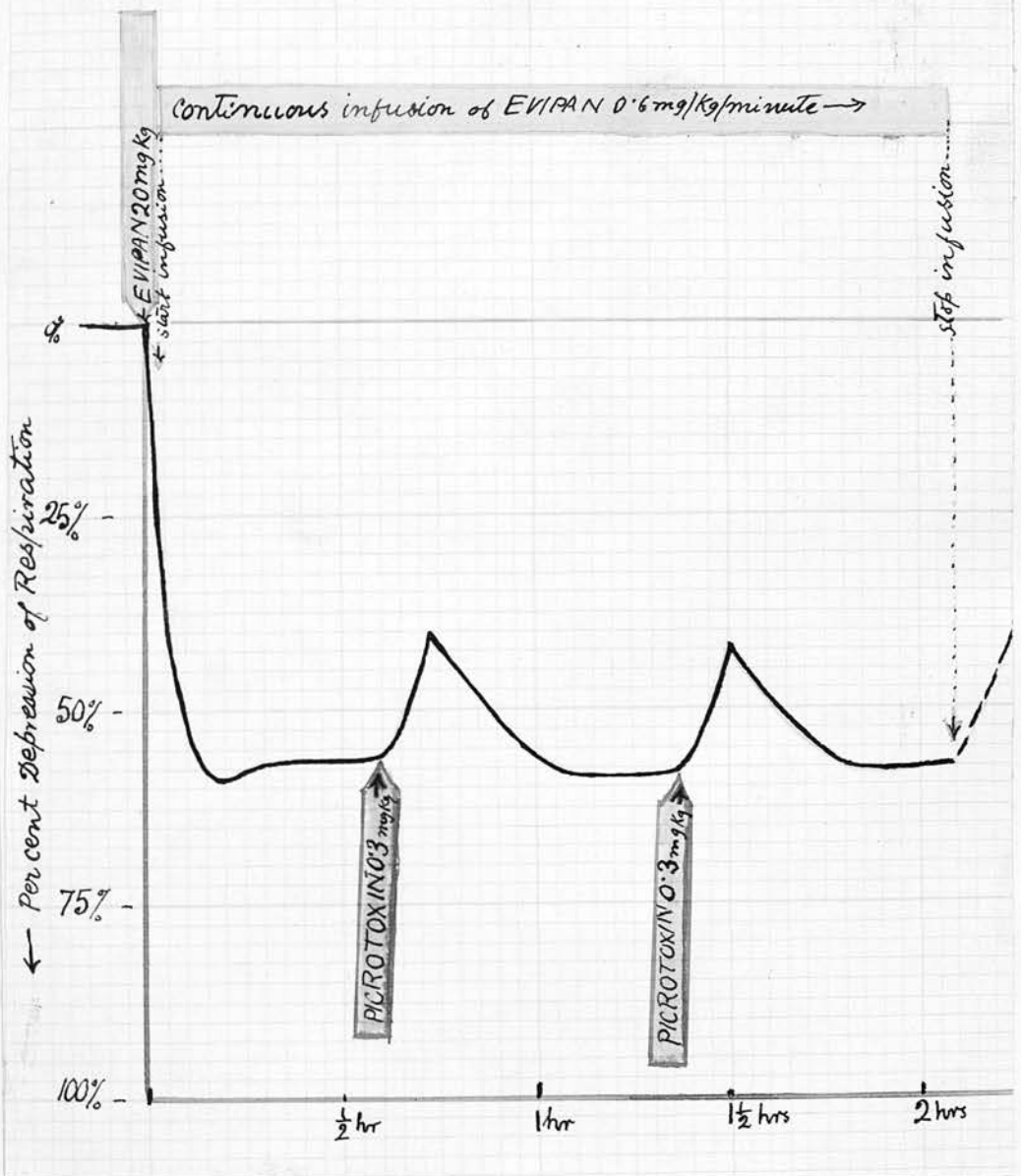


Fig. 13. Respiratory curve of an experiment with two doses of picrotoxin each 0.3 mg./kg. given to a rabbit receiving 0.6 mg./kg./min. of evipan following an initial dose of 20 mg./kg.

The result indicates therefore that the picrotoxin is reduced to 2/3 in 30 minutes. This corresponds to a destruction of about 1.6 per cent. per minute.

A second dose of picrotoxin given about an hour after the first produced an identical response, a fact which supports the view that the picrotoxin is completely detoxicated in the body in a fairly short time.

Picrotoxin was found to be an extremely powerful stimulant even when the respiratory centre was almost completely paralysed. This effect is shown in Fig. 14.(a) and (b). The animal almost stopped breathing and the continuous infusion of evipan was stopped. Two doses of coramine (10 mg. and 20 mg./kg.) produced no benefit. But 0.3 mg./kg. picrotoxin produced an improvement whilst 0.4 mg./kg. picrotoxin given shortly afterwards restored the respiration nearly to normal.

This instance shows the marked superiority of picrotoxin over other analeptics, a subject which will be discussed later.

Fig. 14 /

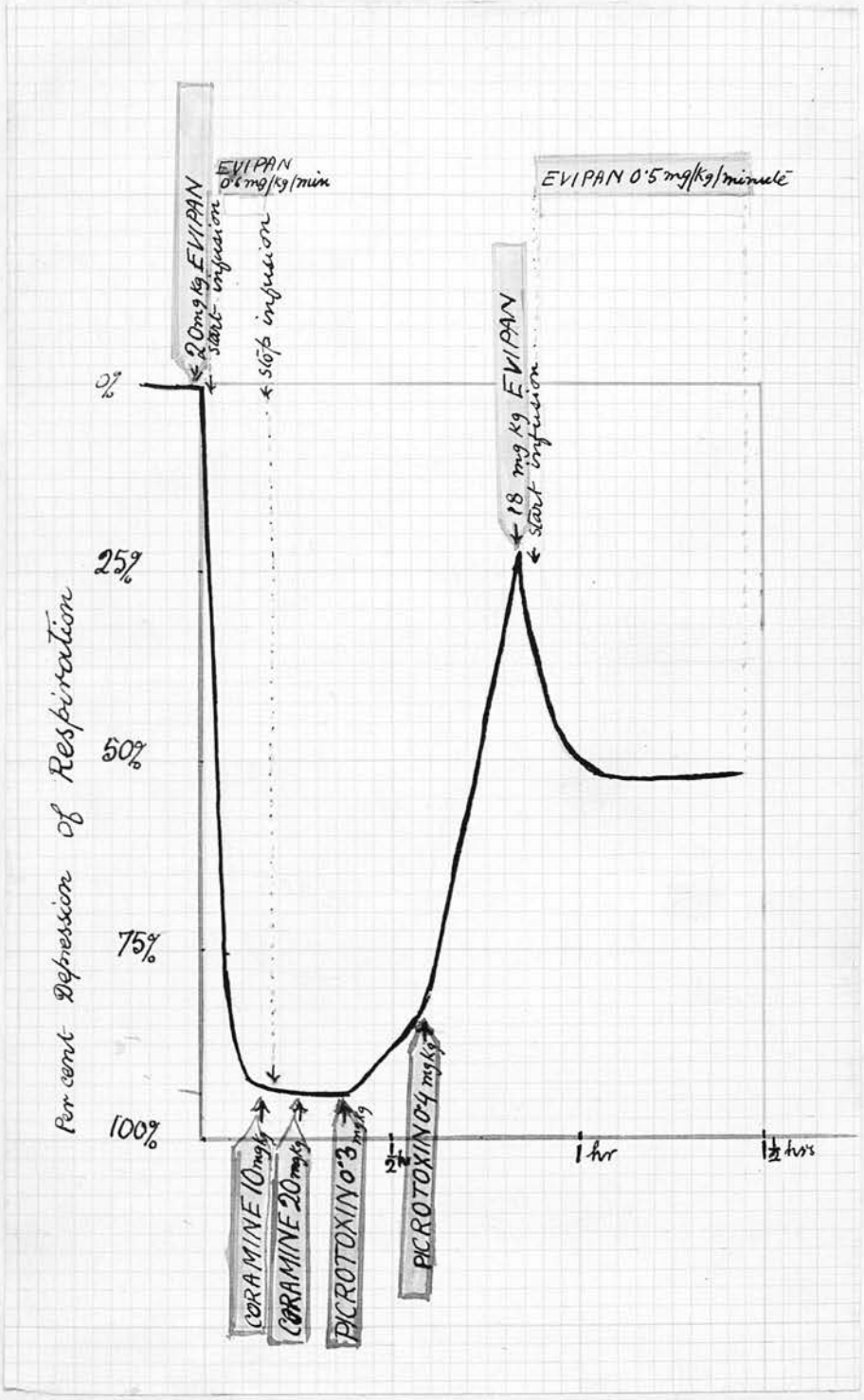


Fig. 14(a). Respiratory curve of a case of severe respiratory depression after evipan. Coramine 30 mg./kg. failed to improve the condition. Picrotoxin 0.3 mg./kg. followed by 0.4 mg./kg. restored the respiratory activity to nearly normal.

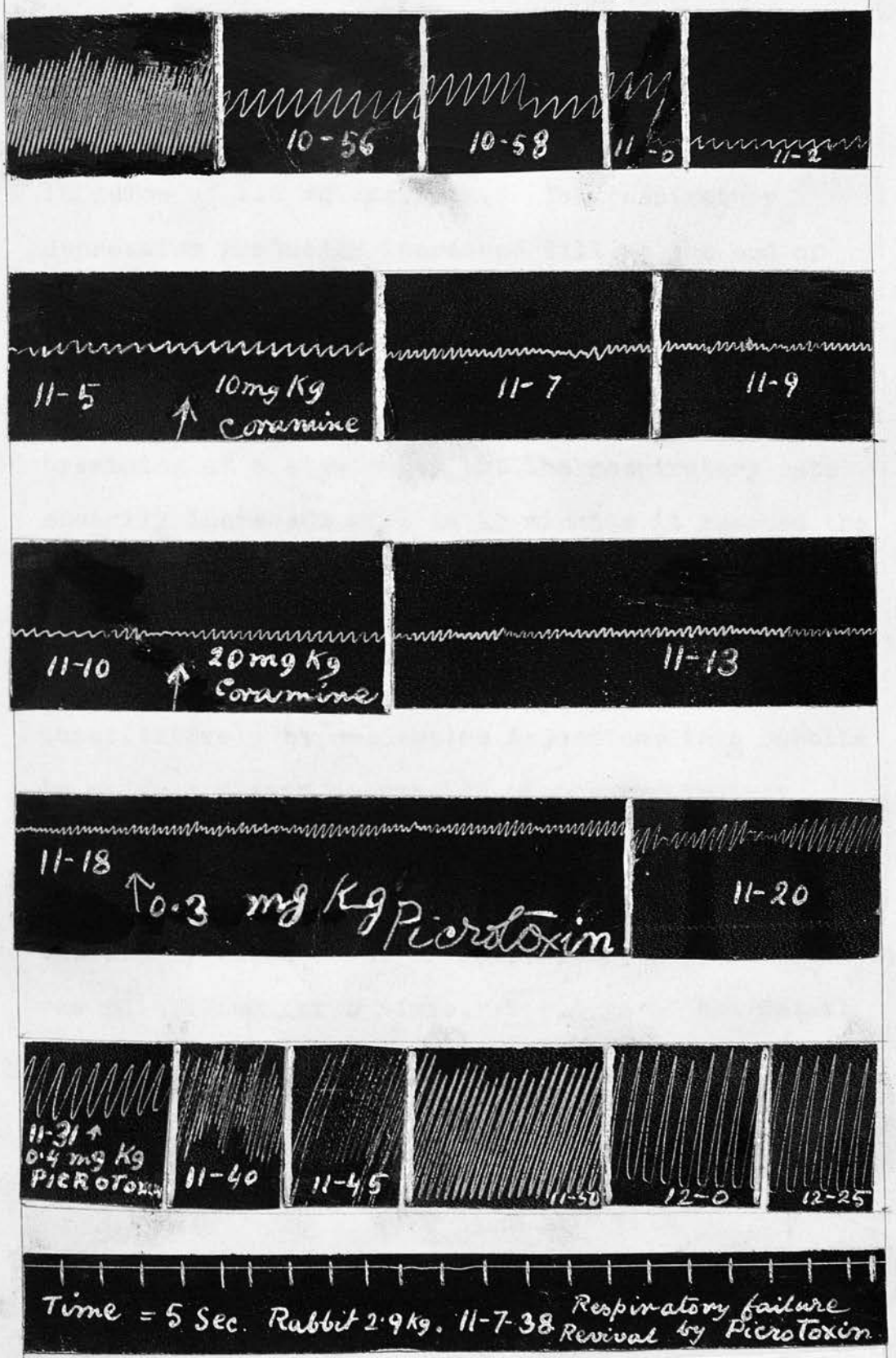


Fig. 14(b). Portions of tracing of the experiment represented by the curve in Fig. 14(a).

In another case a rabbit had an initial dose of 10 mg./kg. evipan followed by a continuous infusion of 1.5 mg./kg./min. The respiratory depression gradually increased till at the end of $1\frac{3}{4}$ hours the respiration stopped completely. Evipan infusion was now stopped and a dose of 1.2 mg./kg. of picrotoxin given.Soon the animal restarted breathing at a slow rate, but the respiratory rate steadily increased till in 10 minutes it reached the normal level. (Fig. 15).

Fig. 16(a) shows the manner in which the action of different analeptics can be compared quantitatively by successive injections into rabbits in which a steady depression of the respiratory centre is maintained by continuous infusion of evipan. The remarkable feature of this figure is the steady basic level of respiratory depression which was maintained for 5 hours. Portions of the actual tracing of this experiment are shown in Fig. 16(b).

Figs. 15 and 16/

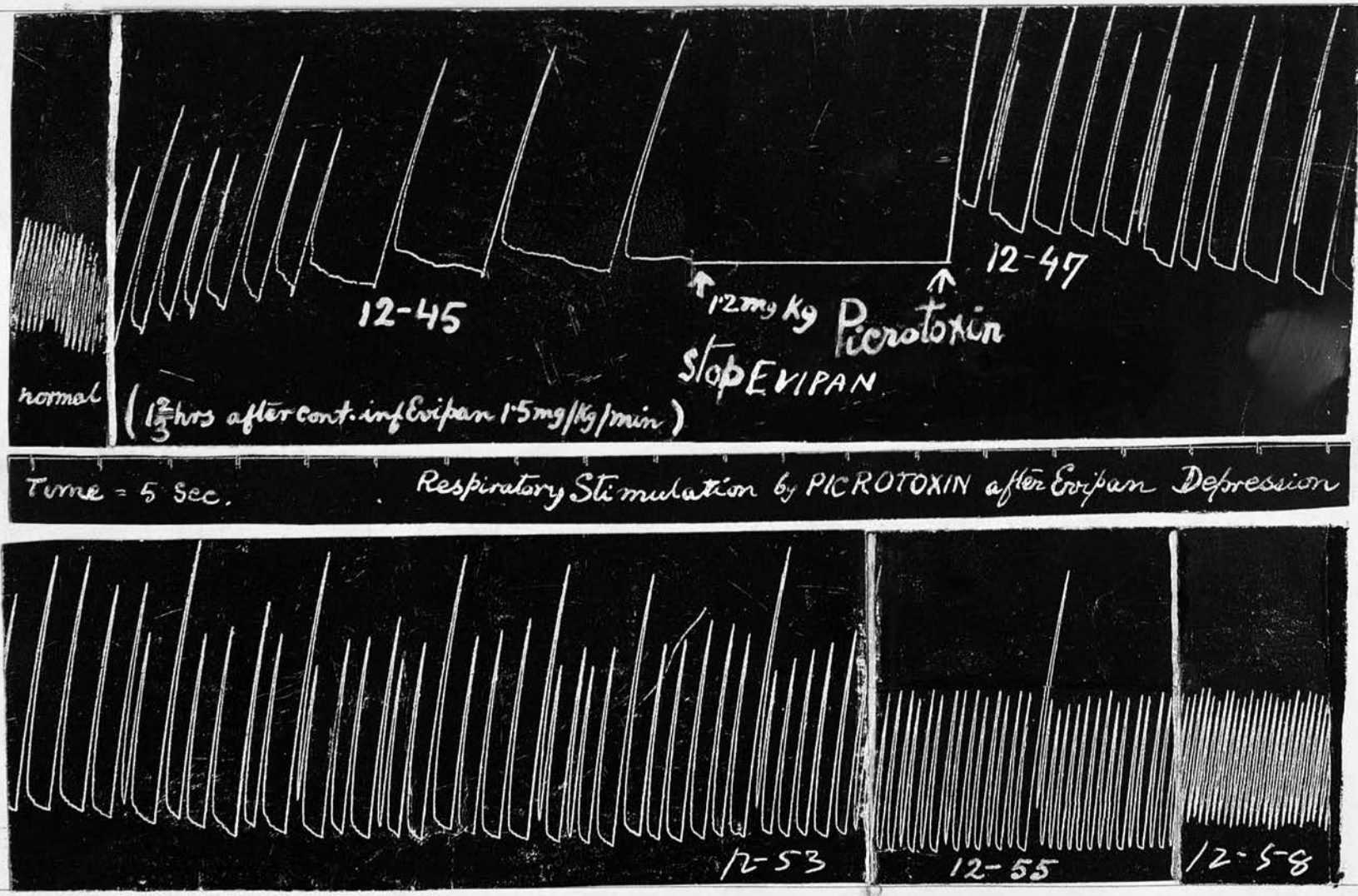


Fig. 15. ~~Blood pressure record~~ Respiration of a rabbit that received 15 mg./kg. evipan followed by 1.5 mg./kg./min. After 1 hour respiration stopped. Evipan infusion was discontinued and 1.2 mg./kg. of picrotoxin, injected i.v. restored the respiratory activity to nearly normal in about 10 min.

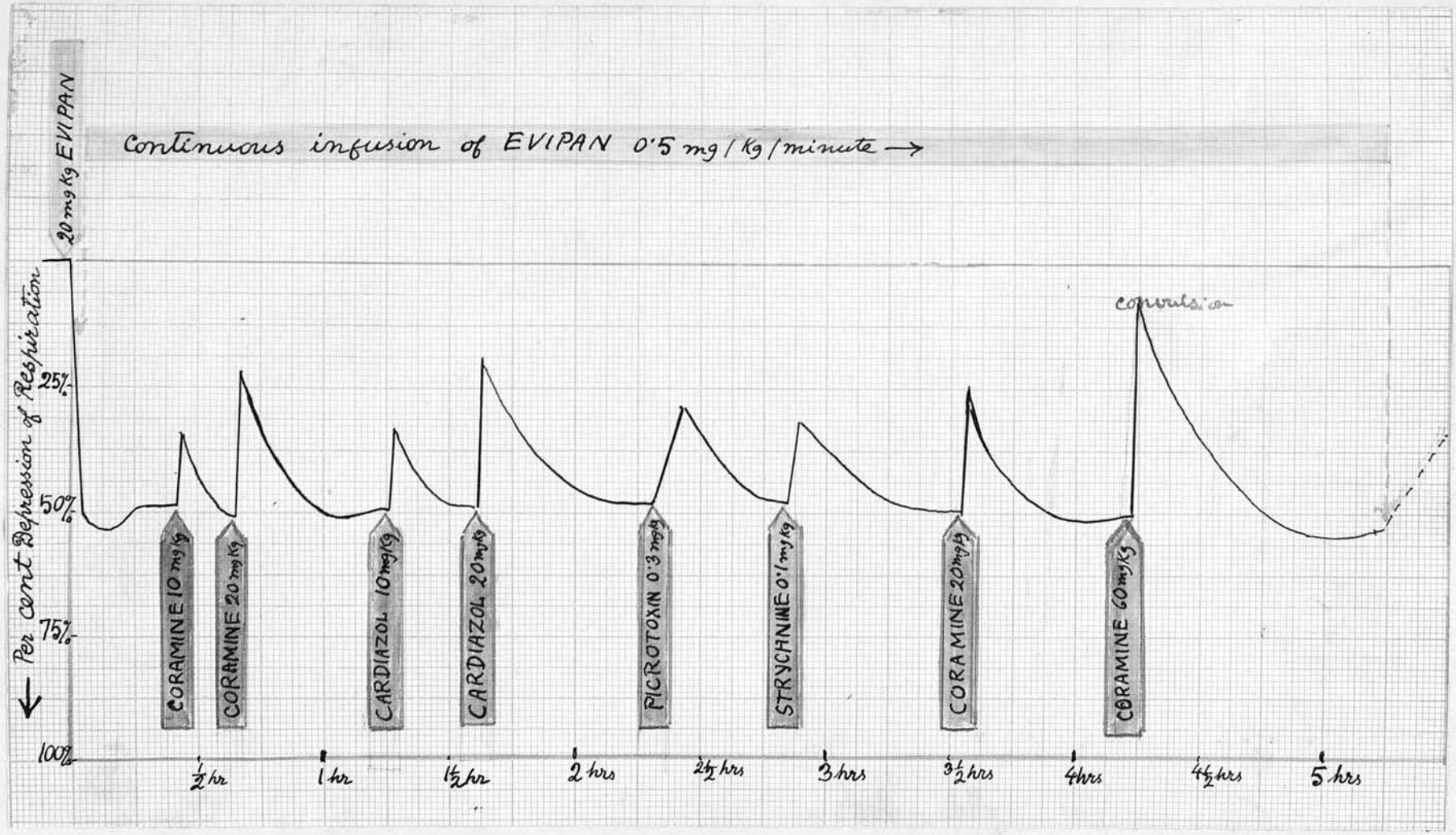


Fig. 16. Respiratory curve of an experiment showing the intensity and the duration of action of different analeptics compared. Basic depression maintained by 0.5 mg./kg./min. of evipan.

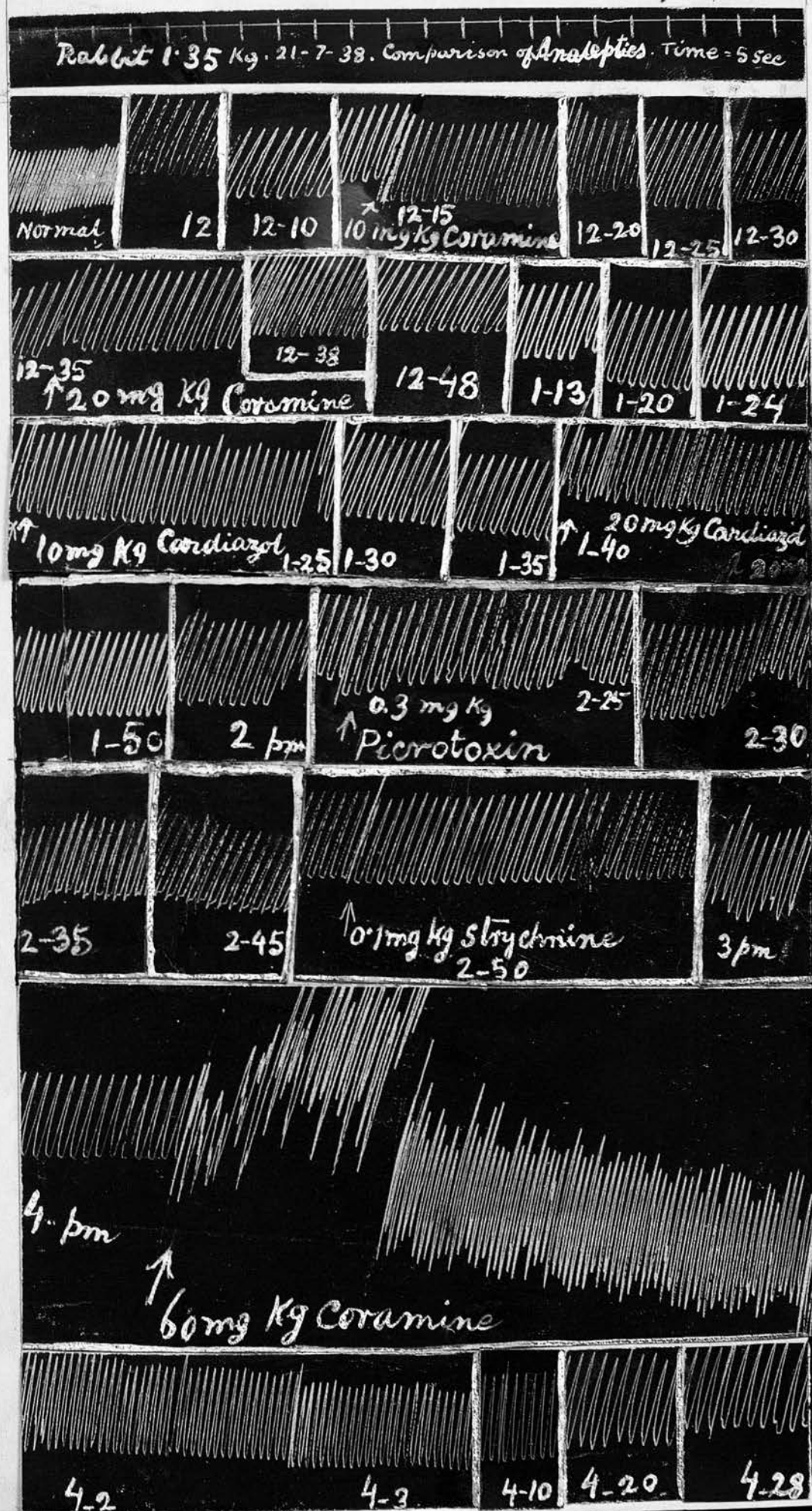


Fig. 16(a). Portions of tracing of the experiment represented by the curve in Fig. 16.

(ii) The antagonism between continuous intravenous infusion of evipan and of picrotoxin.

Fig. 17 shows an example of the results obtained by this method. A steady depression was produced by evipan. Partial recovery was produced by 0.3 mg./kg. picrotoxin and this recovery was maintained at a nearly constant level by continuous intravenous infusion of 0.004 mg. picrotoxin per minute. This result indicates that with this rate of infusion, the rate of destruction of picrotoxin in the body, nearly balanced the rate of infusion. This indicates a destruction of 0.004/0.3 or 1/75 per minute of the drug present.

More striking results were obtained when infusions of evipan were given which would have caused respiratory arrest if not antagonised by picrotoxin.

Fig. 17 /

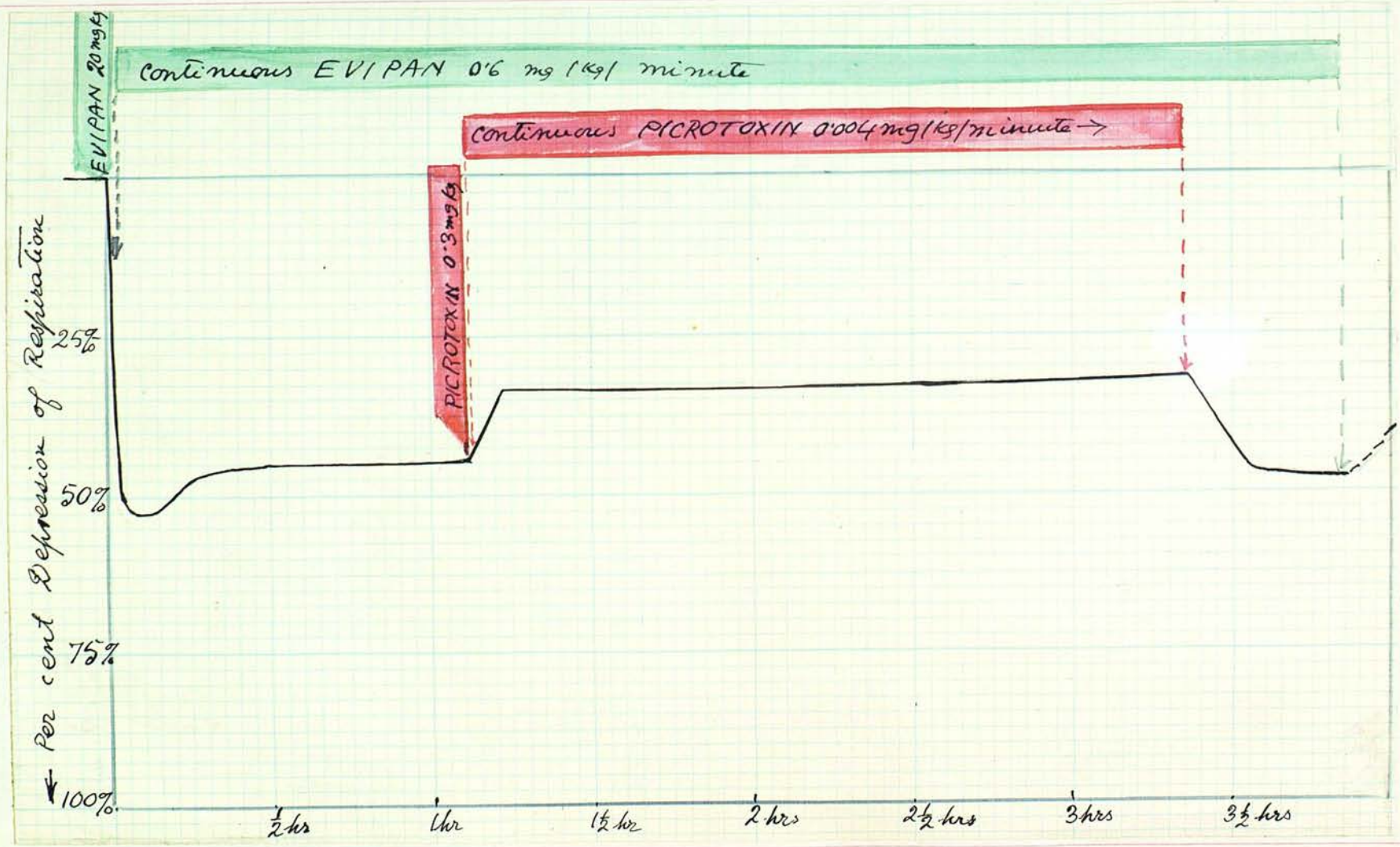


Fig. 17. Continuous infusion of 0.004 mg./kg./min. of picrotoxin following 0.3 mg. of picrotoxin was superimposed on steady depression caused by 0.6 mg./kg./min. of evipan after an initial dose of 20 mg./kg. evipan. The drugs almost balanced.

Fig. 18 shows an example of this type. In this case 0.7 mg./kg. picrotoxin with a continuous infusion of 0.008 mg./kg./min. produced a steady level of partial recovery. This indicates a destruction of 0.008/0.7 = 1/87 per minute of the drug present.

Fig. 19 shows that after 0.9 mg./kg. picrotoxin an infusion of 0.006 mg./kg./min. did not maintain the initial recovery. Hence the amount destroyed was more than 0.006/0.9 = 1/150 per minute.

Fig. 20 indicates a rate of destruction of 0.015/0.7 = 1/48 per minute.

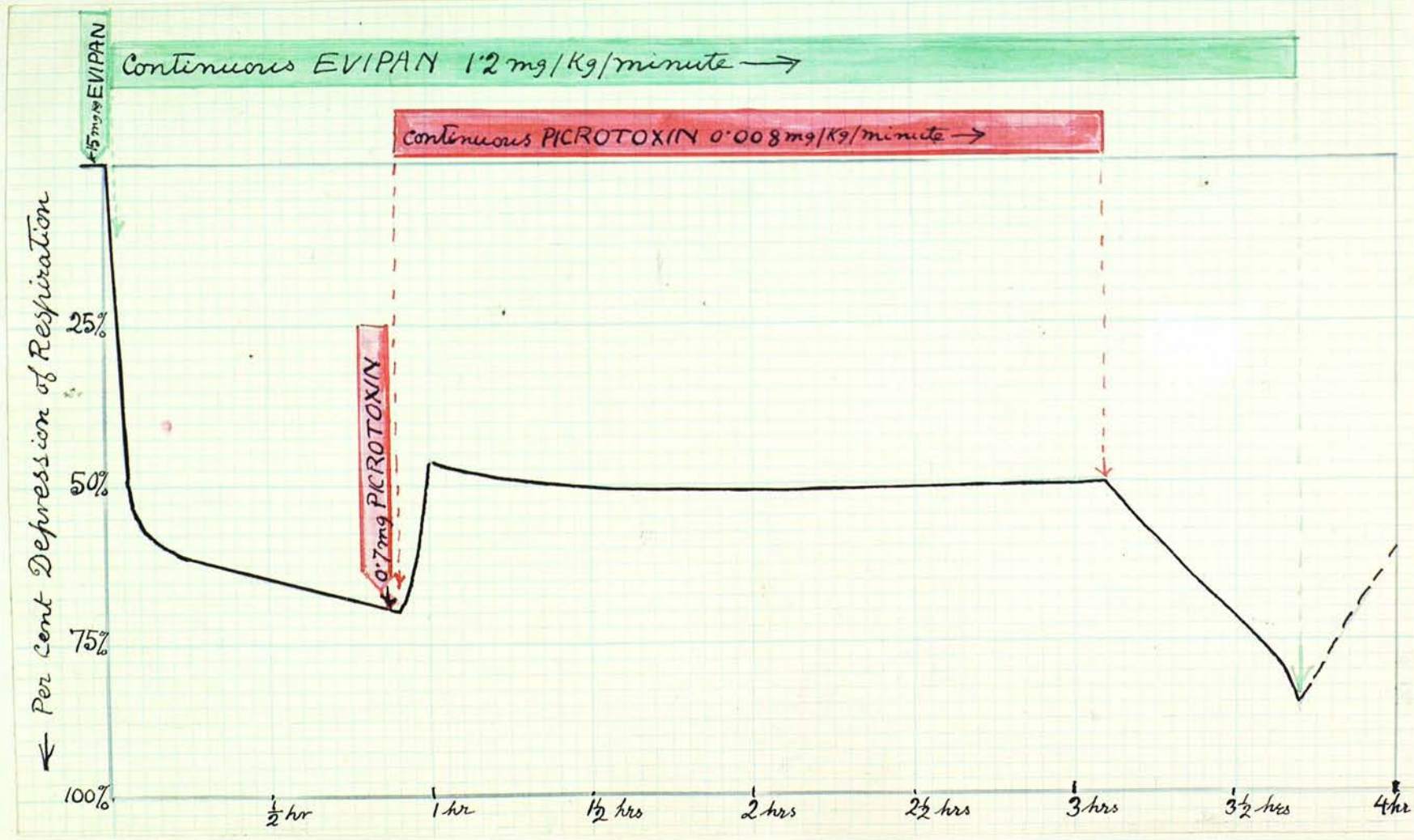


Fig. 18. 15 mg./kg. of evipan followed by 1.2 mg./kg./min. of evipan was causing an increasing depth of depression. 0.7 mg. of picrotoxin raised the respiratory level while a continuous infusion of 0.008 mg./kg./min. of picrotoxin kept it steady for 2 hours. After stopping the infusion the depth of depression was rapidly increasing.

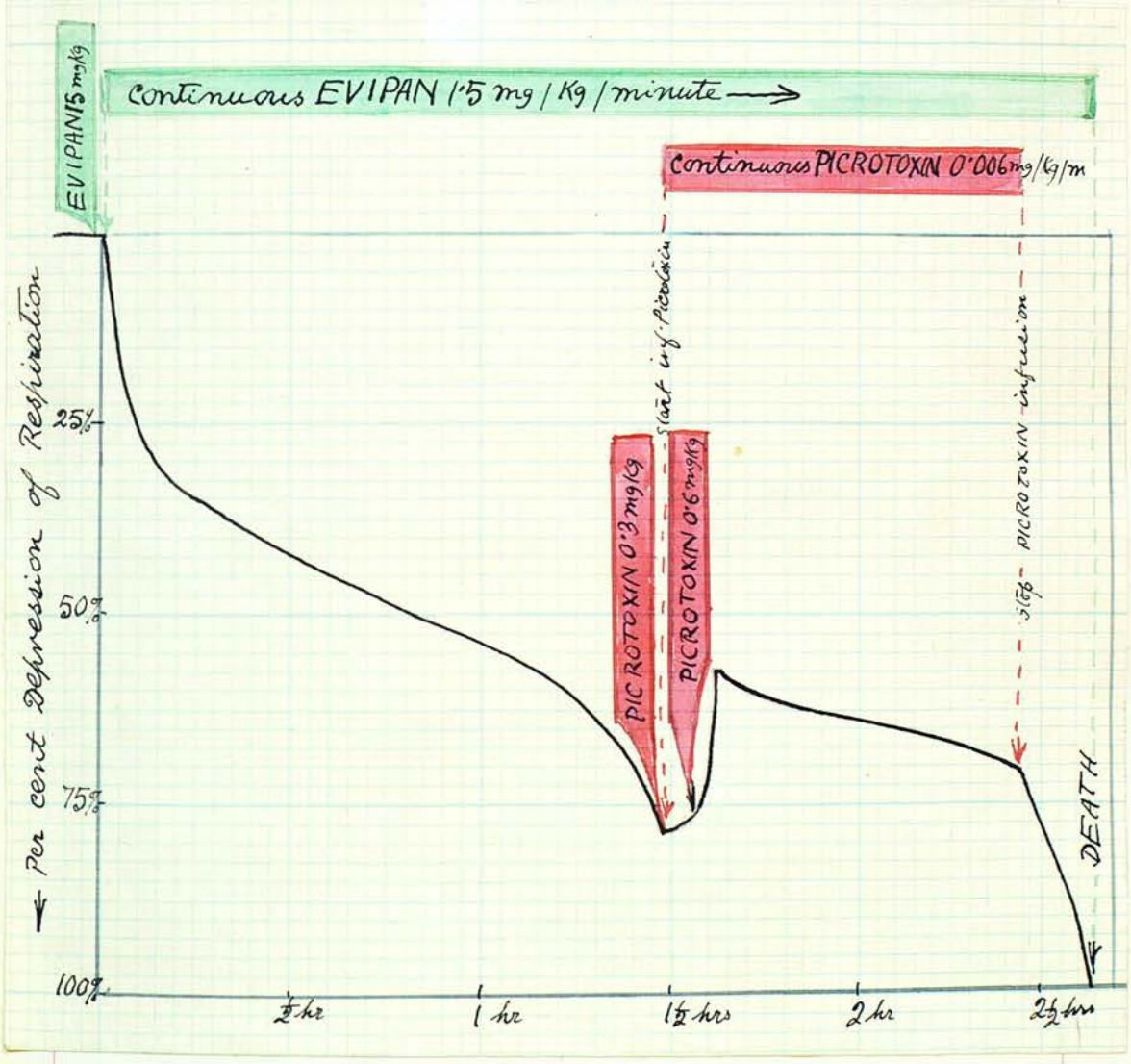


Fig. 19. An initial dose of 15 mg./kg. of evipan followed by 1.5 mg./kg./min. of evipan was causing a rapidly increasing depression. 0.3 and 0.6 (= 0.9) mg./kg. of picrotoxin caused an increase of respiratory activity but 0.006 mg./kg./min. of picrotoxin could not keep it up. On stopping continuous picrotoxin the depression increased so rapidly that the animal died in 10 minutes.

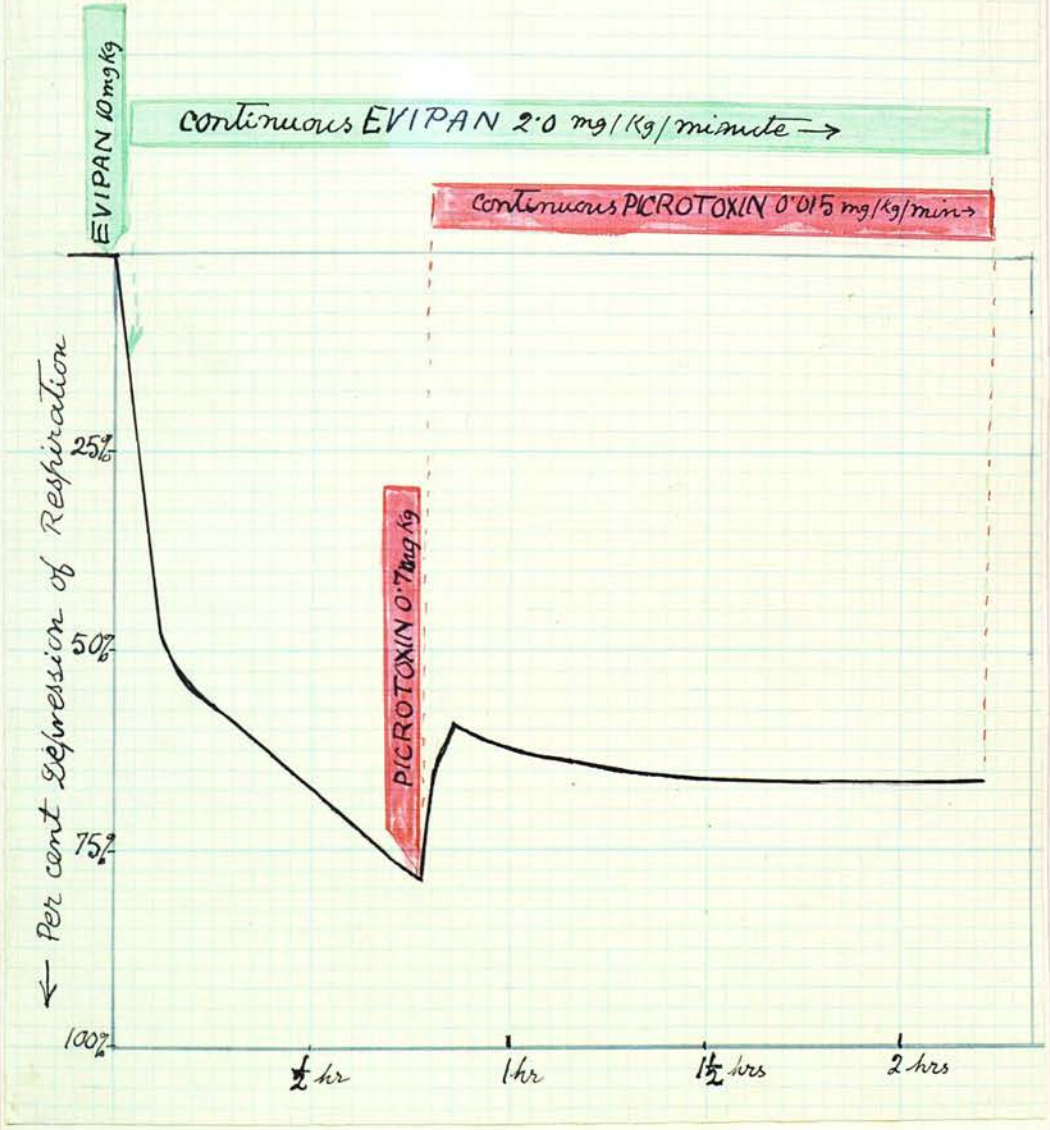


Fig. 20. 2 mg./kg./min. of evipan following an initial dose of 10 mg./kg. was producing a rapidly increasing depression. 0.7 mg. of picrotoxin improved the condition and 0.015 mg./kg./min. of picrotoxin kept it up almost at a steady level.

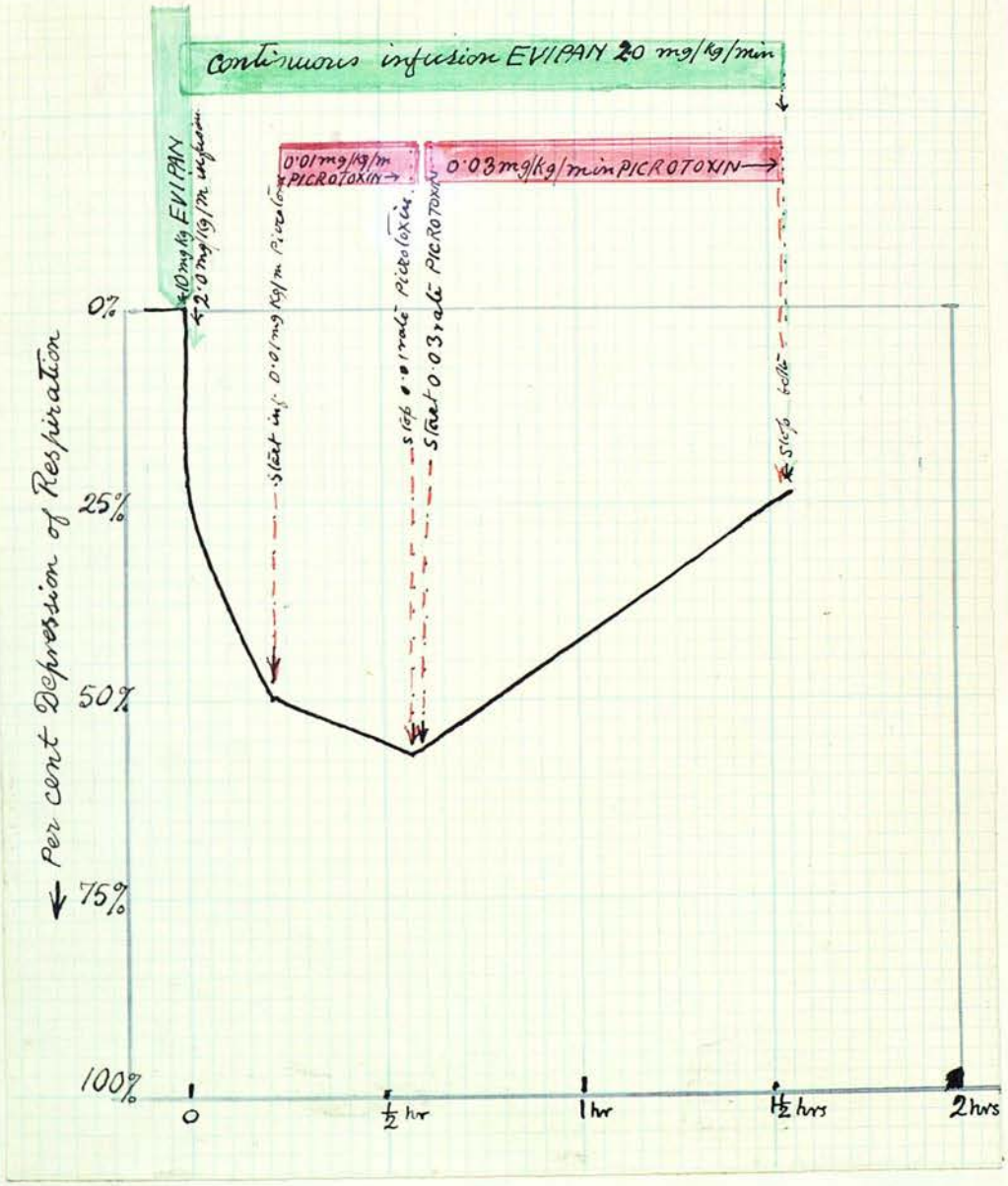


Fig. 21. Depression was rapidly increasing under 2.0 mg./kg./min. following 10 mg./kg. of evipan. A continuous infusion of picrotoxin at the rate of 0.01 mg./kg./min. checked the progress of the depression but could not wholly control it. After a while picrotoxin was infused at the rate of 0.03 mg./kg./min. This proved too strong for the evipan and caused a steady increase of the respiratory activity in spite of the evipan infusion.

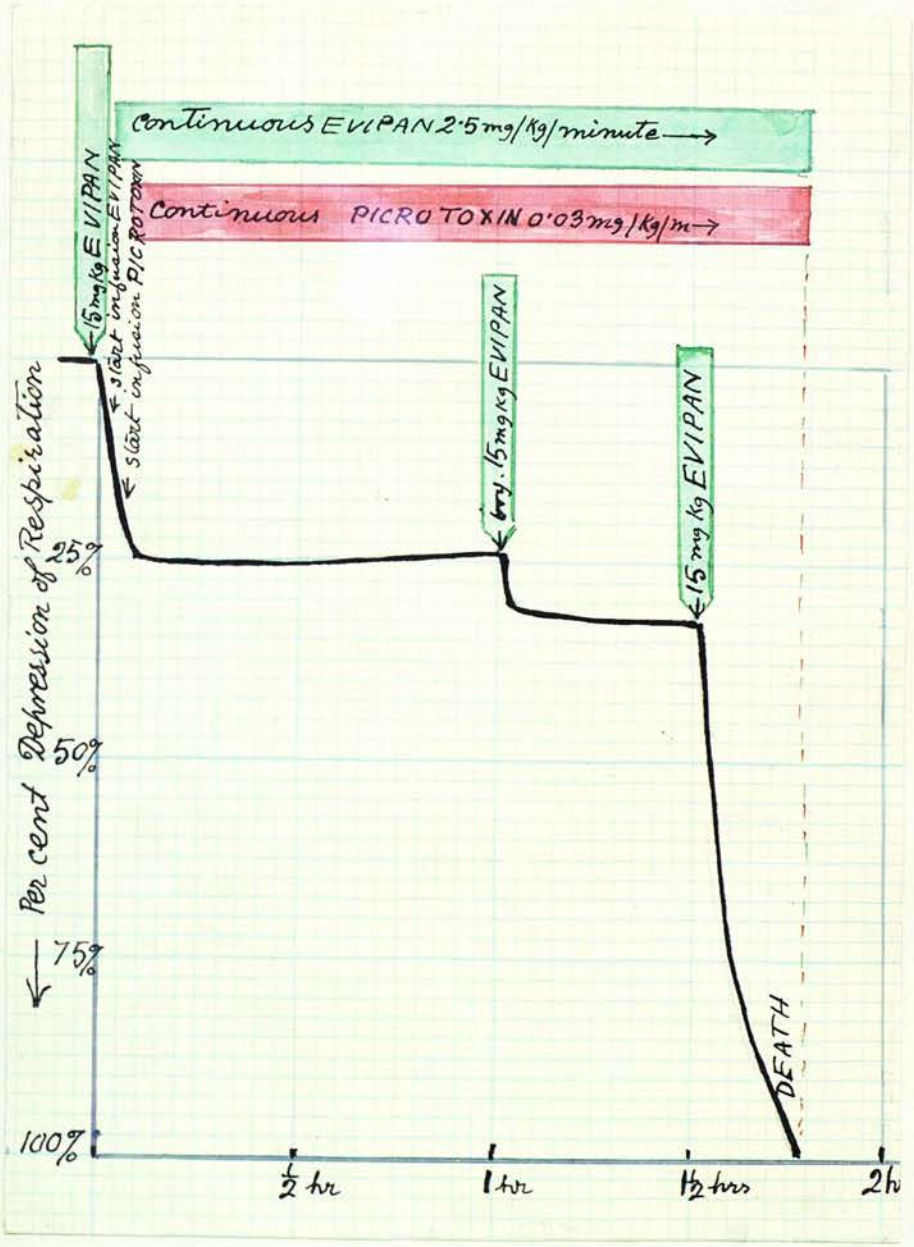


Fig. 22. A depression was caused by a dose of 15 mg./kg. of evipan. Then a continuous infusion of 2.5 mg./kg./min. of evipan was started and was soon followed by a continuous infusion of 0.03 mg./kg./min. of picrotoxin. For one hour the depression was steady. Another dose of 15 mg./kg. of evipan given at this point increased the depth of depression, while the simultaneous continuous infusions of evipan and picrotoxin balancing each other kept the new depression level steady. After another 30 min. a further dose of 15 mg./kg. of evipan given i.v. quickly produced serious depression and soon killed the animal. This last dose turned the scale in favour of too high concentration of evipan which was till then being balanced by the picrotoxin infusion.

Discussion.

The results obtained by the author with continuous simultaneous infusion of picrotoxin and evipan are summarised in Table VI.

Table VI.

Per min. dose of continuous evipan in mg.	Per min. dose of continuous picrotoxin in mg.	Duration of simultaneous infusion in hours	Results	Equivalent doses
0.6	0.004	2	Antagonised	0.6 E 0.004 P
1.2	0.008	2	do.	1.2 E 0.008 P
1.5	0.006	¾	Picrotoxin too little to antagonise. 0.01 mg. would probably antagonise	1.5 E 0.01 P
2.0	0.015	1½	Antagonised	2.0 E 0.015 P
2.5	0.03	1½	do.	2.5 E 0.03 P

On plotting the figures of the last column the curve shown in Fig. 23(a) is obtained. If the logs. of the picrotoxin doses are plotted against the corresponding evipan doses, a linear relationship is obtained, as seen in Fig. 23(b). The nature of these results agrees in general with those obtained by Maloney and co-workers (1931) and also by Koppanyi et al. (1936).

Interpretation of the above findings:

Evipan. The results of the experiments with continuous infusion of evipan alone have, as shown in a previous chapter, led to the assumption that about 1/25 to 1/30 of the evipan present is destroyed per minute.

Picrotoxin. In case of picrotoxin a single dose of 0.3 mg./kg. acts for about 20 minutes.

The minimum effective dose was found to be (a) 0.2 mg./kg. in the case of single injections and (b) 0.003 mg./kg./min. in the case of continuous infusions.

A destruction rate of 1/70 per minute would account for this ratio. This corresponds to half destruction in 48 minutes. The rate of detoxication of/

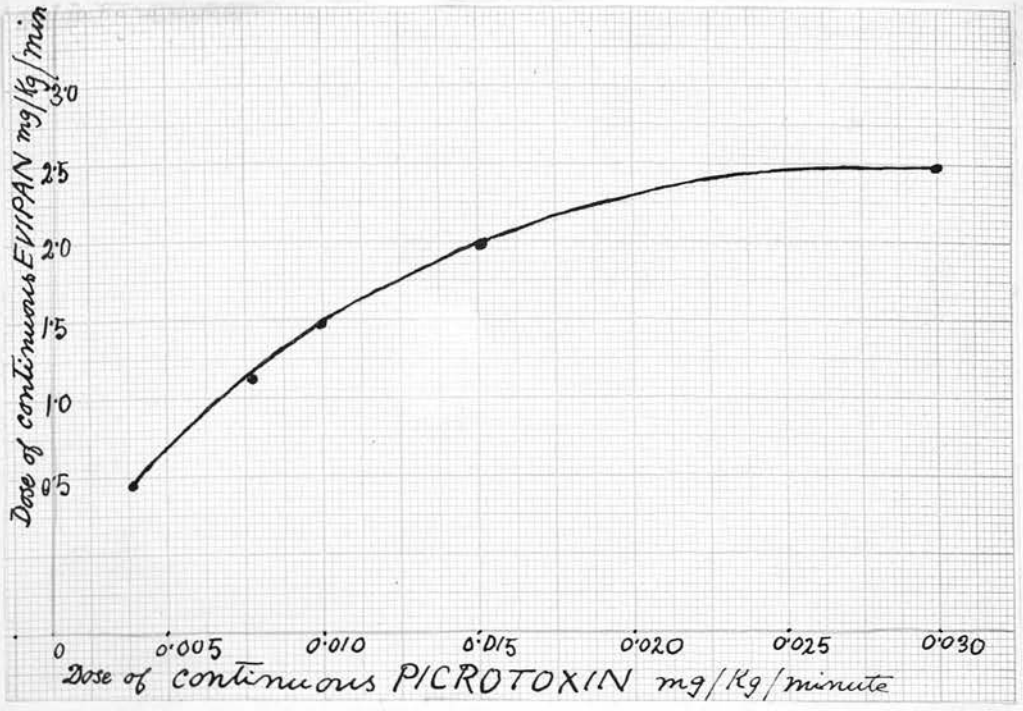


Fig. 23(a). Curve showing relation of the doses of continuous evipan and the antagonising doses of continuous picrotoxin.

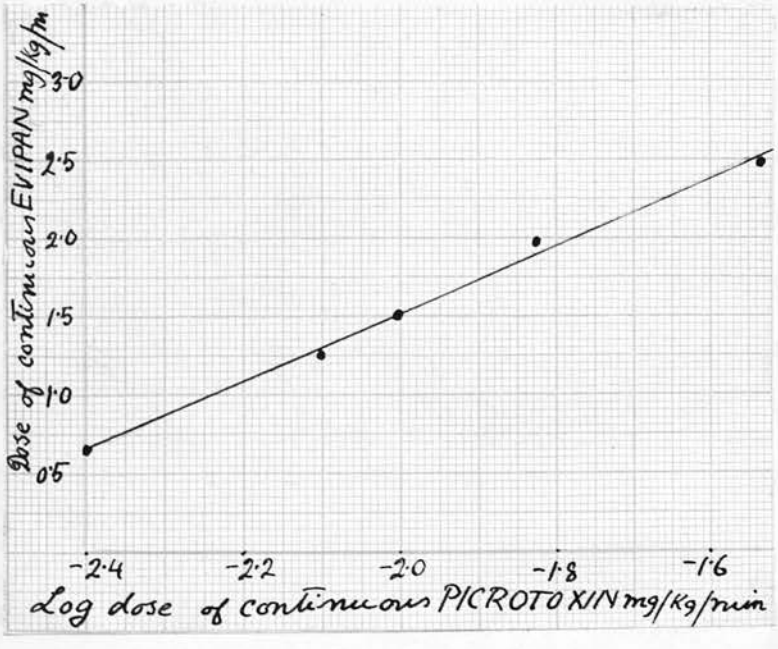


Fig. 23(b). Curve showing the relation of the doses of continuous evipan and the logs. of the antagonising doses of picrotoxin. It may be noted that this practically a straight line.

of picrotoxin is thus about half that of evipan. Hence intravenous infusion produces relatively greater cumulation in the case of picrotoxin.

Estimates of the rate of picrotoxin destruction made above vary from 1/48 to 1/87 per minute. It would appear that between 1 to 2 per cent. of the picrotoxin present is destroyed per minute. This rate is considerably slower than the rate found for evipan (3 to 5 per cent. removed per minute.

In the case of evipan it was possible to produce fairly conclusive proof that a fixed proportion of the drug present was removed per minute. The evidence in the case of picrotoxin is less conclusive, but figures 17 to 20 show that with rates of infusion varying from 0.004 to 0.015 mg./kg./min. it is possible to maintain a steady level of partical recovery of respiratory activity for hours. It is difficult to imagine how such a result could be attained unless a constant portion of the drug present is destroyed per minute, since only this mechanism will result in a constant intravenous infusion producing a steady effect.

Summary.

1. Picrotoxin, as a respiratory analeptic, showed an increase in the amplitude as well as in the rate of respiration. Its action started at once but the maximum effect took some time to develop. Repetition of the same dose produced similar effects.

2. Picrotoxin was steady in its effects at different depths of depression and produced a response even in extremely depressed conditions.

3. A dose of 0.3 mg./kg. of picrotoxin showed the same intensity of action as 10 mg./kg. of cardiazol but picrotoxin had a longer duration.

4. When a suitable dose of picrotoxin was continually infused simultaneously with a lethal dose of continuous evipan, the evipan was fully antagonised. The dose of evipan that could thus be antagonised varied with the log. dose of the picrotoxin required.

VI. CORAMINE.

The analeptic activity of coramine was tested in the same manner as was described in the case of picrotoxin. A steady depression of respiration was produced in the rabbit by continuous intravenous infusion of evipan, and the stimulant action of coramine on respiration was estimated firstly by single injections and secondly by continuous infusion.

I.(a) Effect of single doses of coramine.

The result of an experiment of this group is shown in Fig. 24. The rabbit was receiving a continuous infusion of 0.5 mg./kg./min. of evipan following an initial dose of 15 mg./kg. of evipan. When the depression was steady, a dose of 20 mg./kg. of coramine was given. There was a prompt increase in the respiratory activity. This is shown by the quick rise in the respiratory curve; but the sharp descent of the curve indicated a rapid fall in the respiratory activity, and a short-lived action. The actual tracing showed an increase in the rate of the respiration rather than an increase in the excursion of each respiratory movement.

Fig. 24/

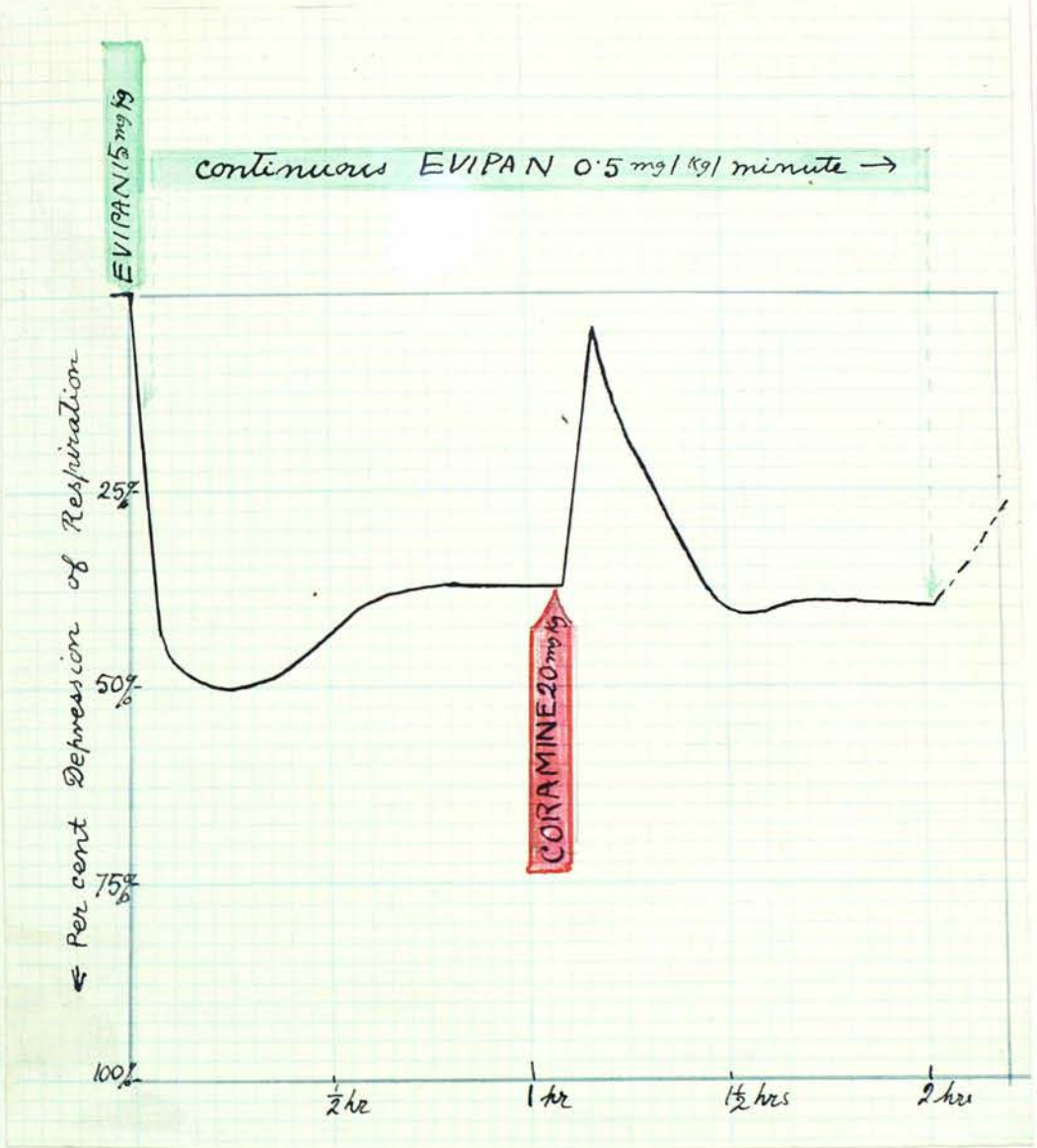


Fig. 24. Respiratory curve of an experiment showing the intensity and duration of action of 20 mg./kg. of coramine given to a rabbit under continuous evipan 0.5 mg./kg./min. following an initial dose of 15 mg./kg. of evipan.

The durations of action of various doses of coramine as obtained in different experiments are given in a tabular form below. (Table VII)

Table VII.

Dose of coramine per kg.	Log. of the dose	Durations in min. as obtained in different expts.	Average duration in min.
5 mg.	0.69	5	5
10 mg.	1.00	12,11,9,13	11.5
40 mg.	1.60	22, 24	23
60 mg.	177	28	28

Plotting these doses with the average duration gives the curve shown in Fig. 25(a). If the logs. of these doses are plotted with the corresponding average duration, a straight line is obtained (Fig. 25(b). This indicates that the duration of coramine action varies with the log. dose of coramine.

Fig. 25(a) and (b) /

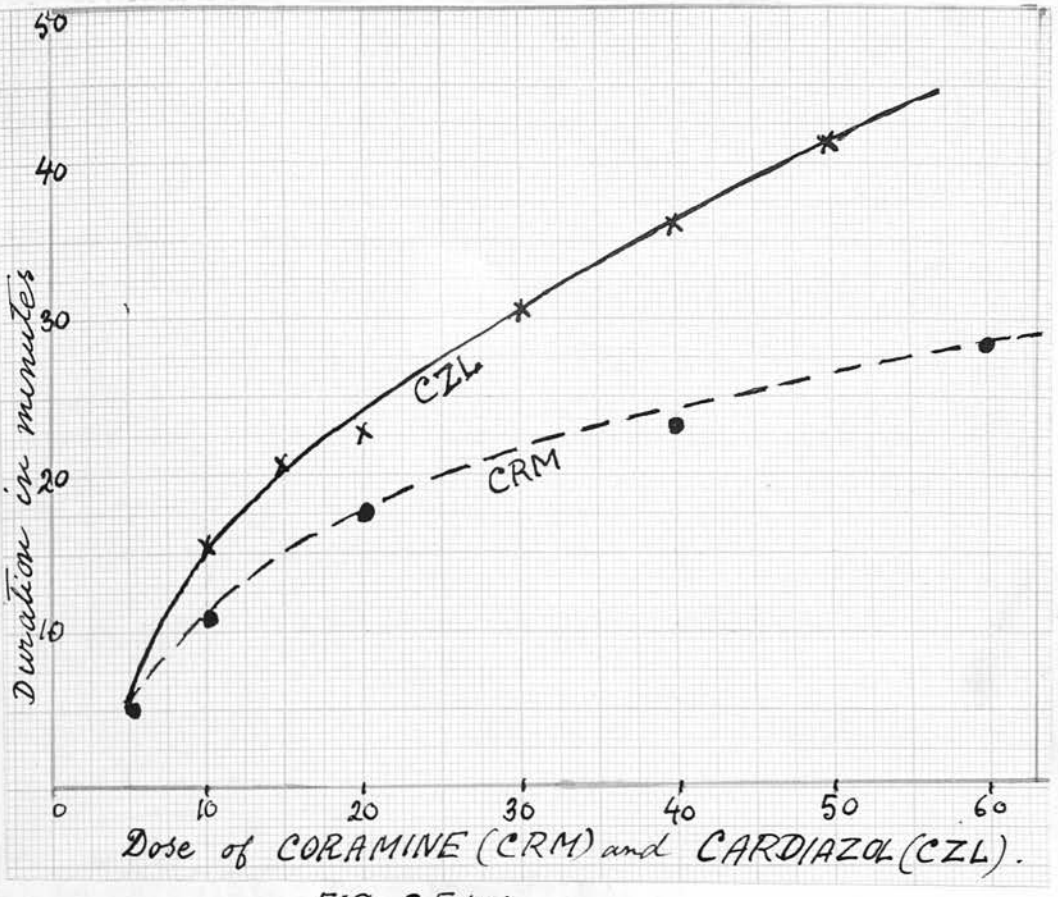


FIG. 25 (a)

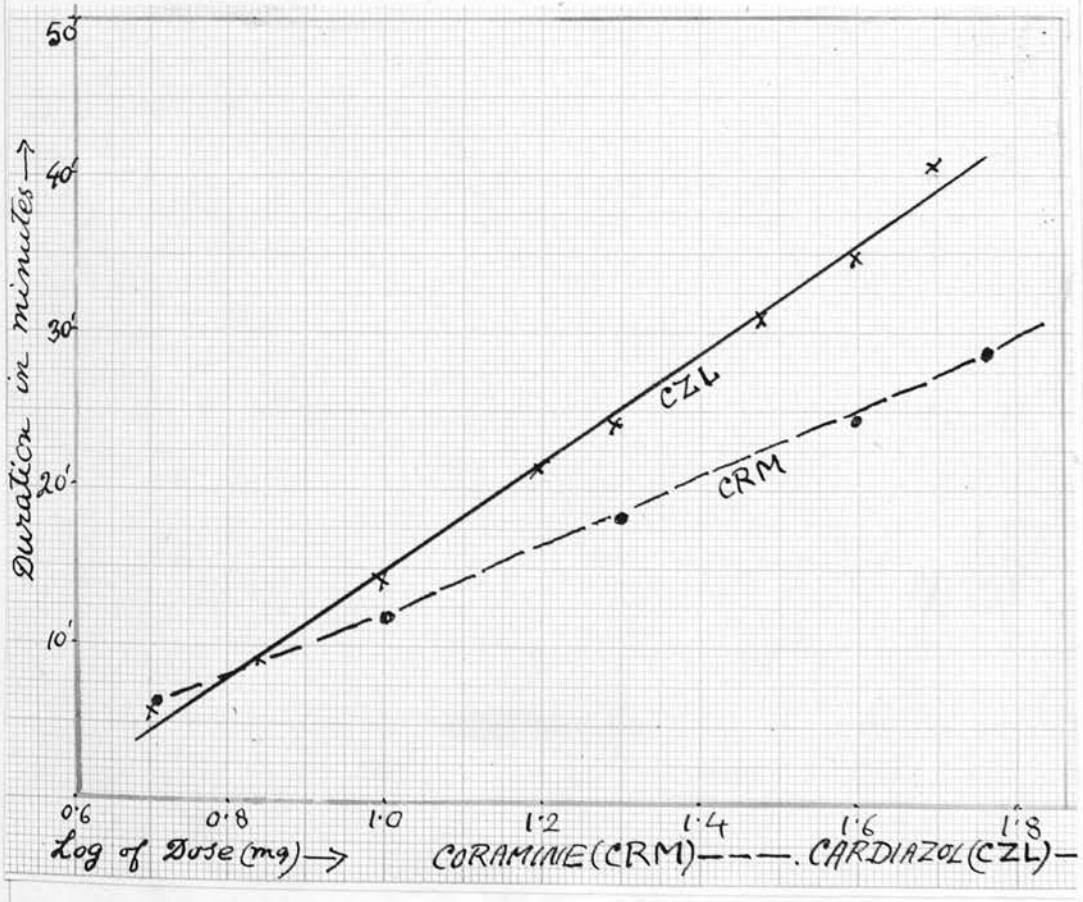


FIG. 25 (b)

Fig. 25 (a). Curves showing the duration of action of coramine (broken line - - -) and of cardiazol (continuous line (——) .

Fig. 25 (b). Curves showing the relation between the durations of action and logarithm of the doses of coramine (broken line - - -) and of cardiazol (continuous line (——) .

Extrapolation of the curves indicates that 3 mg./kg. would produce no action. The slope of the curve,when log. dose is plotted against time, corresponds to half destruction (decrease by log. 0.3) in 8 minutes or a loss of 1/10 per minute.

A continuous infusion of 0.25 mg./kg./min. produced an appreciable effect. According to hypothesis already described, this should produce a maximum accumulation of 0.25 x 10 = 2.5 mg./kg. This is just below the minimum effective dose that is calculated from Fig. 25(b).

It is of interest to note that the rate of destruction of coramine (1/10 per minute) is much greater than that of picrotoxin (1/70 per minute).

The analeptic action of coramine was found not to be constant in all cases. In some cases of extreme depression, especially when the depression was rapidly progressing, coramine failed to produce any change. An example of this can be seen in Fig. 26. In this case, 20 mg./kg. of coramine, administered on a steady depression maintained by 0.5 mg./kg. of evipan, produced a brisk response. But soon the respiratory activity began to decline very rapidly, as shown by the steep downward course of the curve.

Fig. 26/

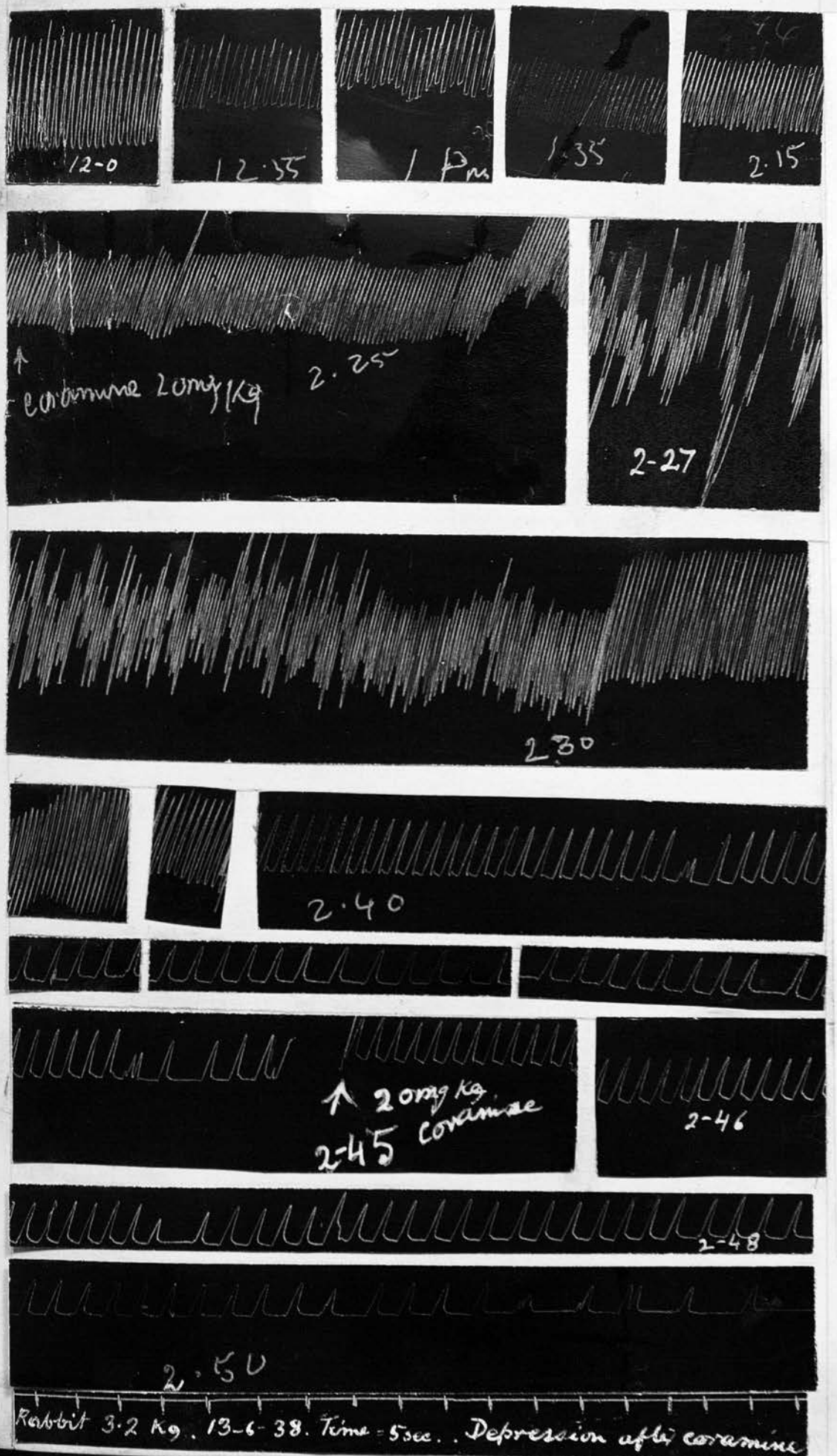


Fig. 26(a). Portions of tracing of experiment represented by the curve in Fig. 26.

On observing this, the continuous evipan was stopped and another 20 mg./kg. of coramine was injected intravenously. In spite of this second dose, the downward course of the curve continued and the animal died in ten minutes. It seems that the rapid fall after the first injection might have been caused by some sort of hypersensitiveness of the animal to an intermediary breakdown product of coramine, but the point of special interest is the failure of the second dose to produce any effect whatsoever. A similar behaviour of coramine was observed in a few other experiments also.

I. (b). Comparison with other analeptics.

The respiratory curves of three experiments of this group are given in Figs. 16(a), 27 and 28.

Figs. 27 and 28 /

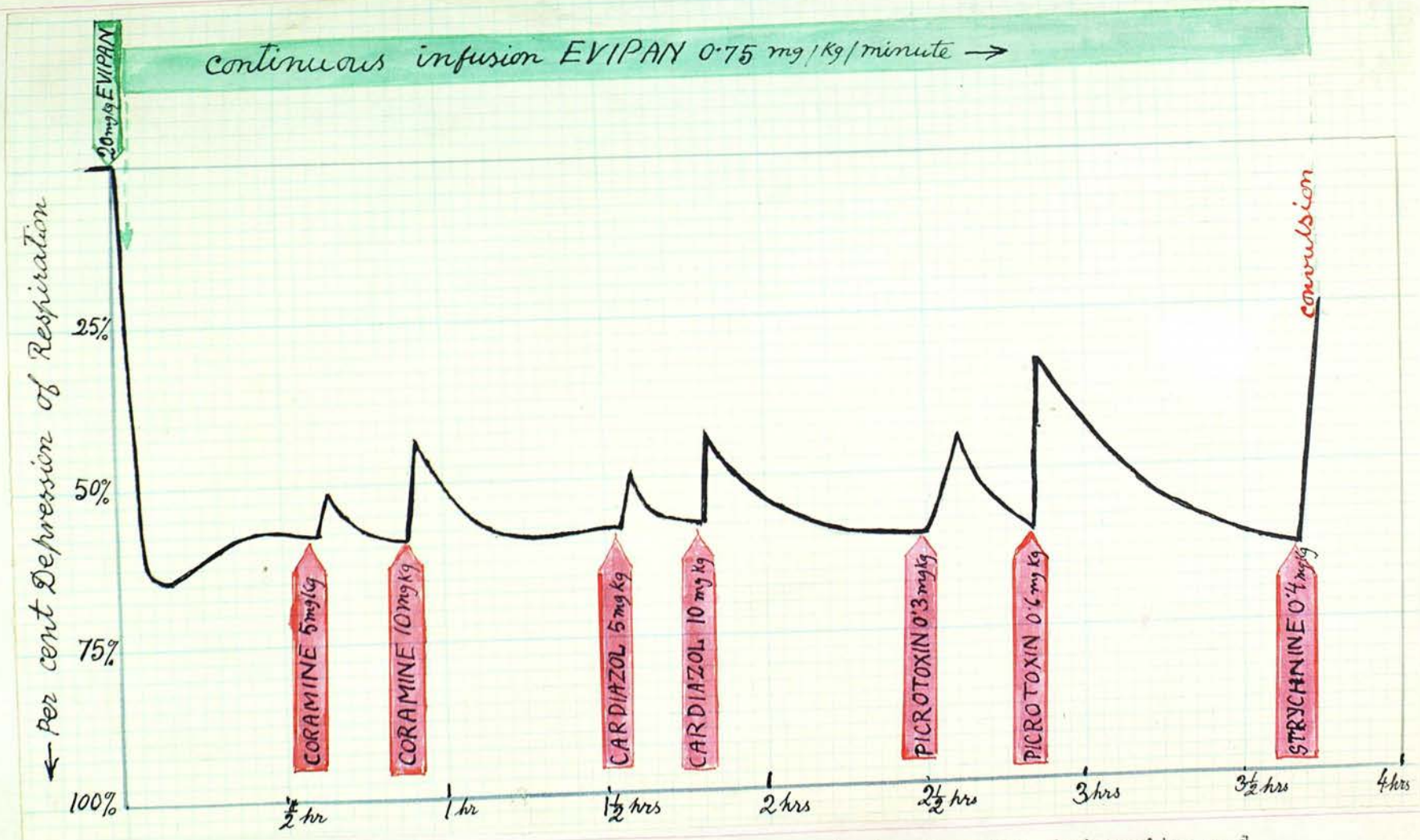


Fig. 27. Respiratory curve of an experiment showing the intensity and duration of action of different analeptics. Basic depression maintained by 0.75 mg./kg./min. of evipan.

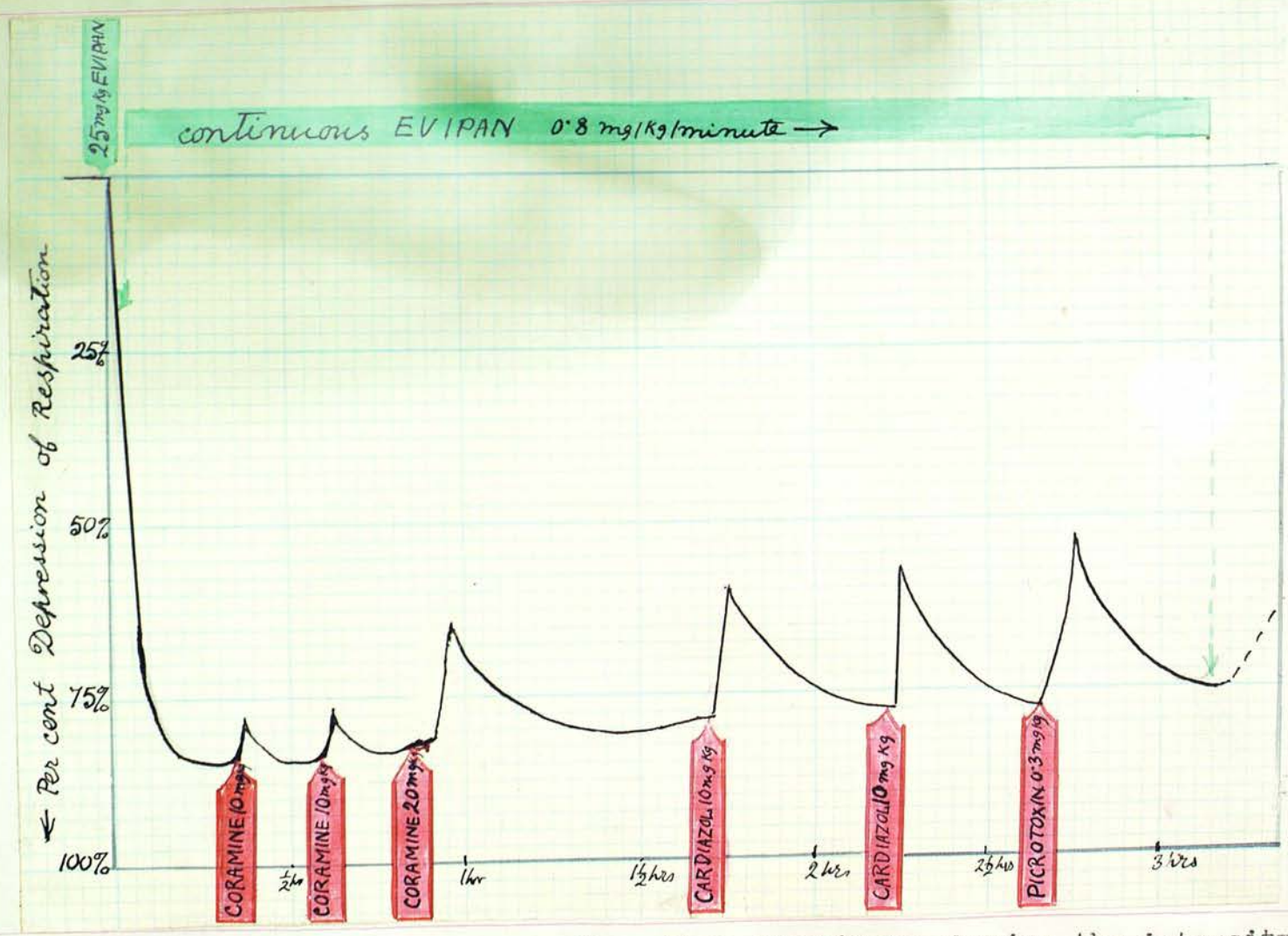


Fig. 28. Respiratory curve of another experiment showing the intensity and duration of action of different analeptics. Basic depression maintained by 0.8 mg./kg./min. of evipan.

In the first one, the depression was at the 50 p.c. level, and the intensity of action of 10 m.g/kg. of coramine was slightly more than that of 10 mg./kg. of cardiazol or 0.3 mg./kg. of picrotoxin, but the duration of the picrotoxin action was longer. In the second one, the depression was between 55 and 60 p.c. and the intensity of the action of 10 mg./kg. coramine was equal to that of 10 mg./kg. of cardiazol or of 0.3 mg./kg. of picrotoxin. In the third one, which had a depression level at about 80 p.c., even the effect of 20 mg./kg. coramine was less than that of 10 mg./kg. of cardiazol or of 0.3 m.g/kg. of picrotoxin. In another experiment (Fig. 14(a)) already reported, the depression line reached the 95 p.c. level, 30 mg./kg. of coramine (in two doses within 5 minutes) failed to produce any effect whereas 0.3 mg. of picrotoxin started improving the animal's condition and another 0.4 mg./kg. (given after 15 minutes) rapidly accelerated the progress and raised the respiratory curve to the 20 p.c. level in a quarter of an hour. Thus it was seen that in conditions/

conditions of deeper depression coramine could not keep up the equiactive dosage relationship with cardiazol or picrotoxin and even failed to produce any effect in extremely depressed conditions.

II. Continuous Infusion of Coramine.

In this group of experiments, after obtaining a steady level of depression with a continuous infusion of evipan, coramine also was given by continuous infusion simultaneously. Below 0.2 mg./kg. of coramine per minute, no change could be seen in the horizontal course of the curve. A dose of 0.25 mg./kg. per minute of continuous coramine caused a very slow rise of the curve as can be seen in Fig. 29, but after a while, in spite of the coramine continuing, the curve began to descend even below the evipan level. On stopping the coramine infusion, the curve slowly rose to the previous level of steady depression and then continued a horizontal course.

Fig. 29 /

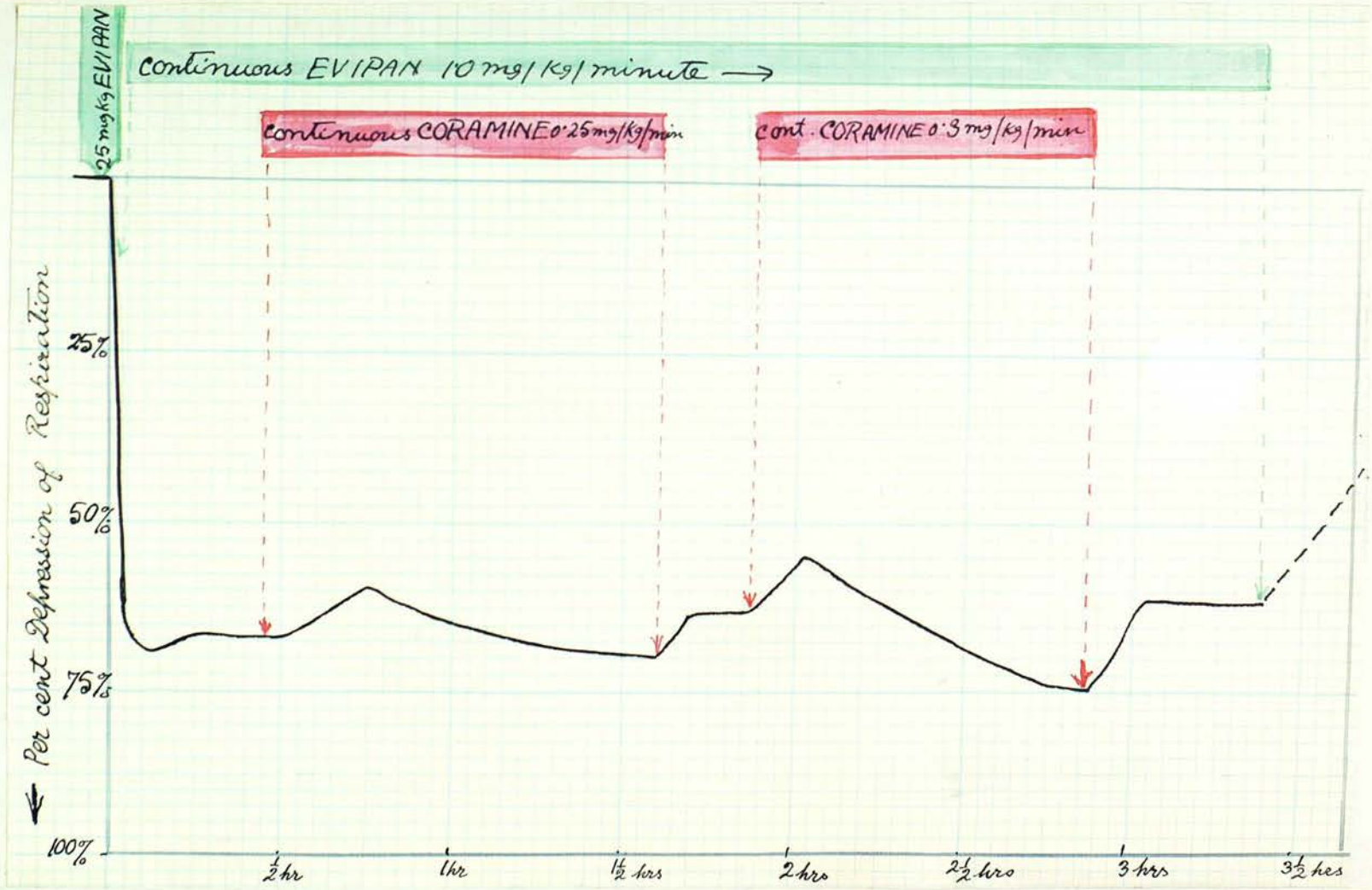


Fig. 29. Respiratory curve showing the effect of continuous coramine. 0.25 mg./kg min. of continuous coramine after an initial stimulation lowered the respiratory activity even below the basic level due to evipan. Respiratory activity increased after stopping continuous coramine. 3 mg./kg./min. of coramine produced similar and more prominent results.

0·3

A bigger dose (0.3 mg./kg./min.) of coramine infusion was again started and this produced a similar and more pronounced effect. After some rise, the curve began to descend and continued to do so till well below the starting level. On stopping coramine infusion, respiration improved till it reached the original evipan level which it followed as long as the evipan infusion was continued. Still clearer was the picture presented in Fig. 30 giving results of a similar experiment.

Fig. 30 /

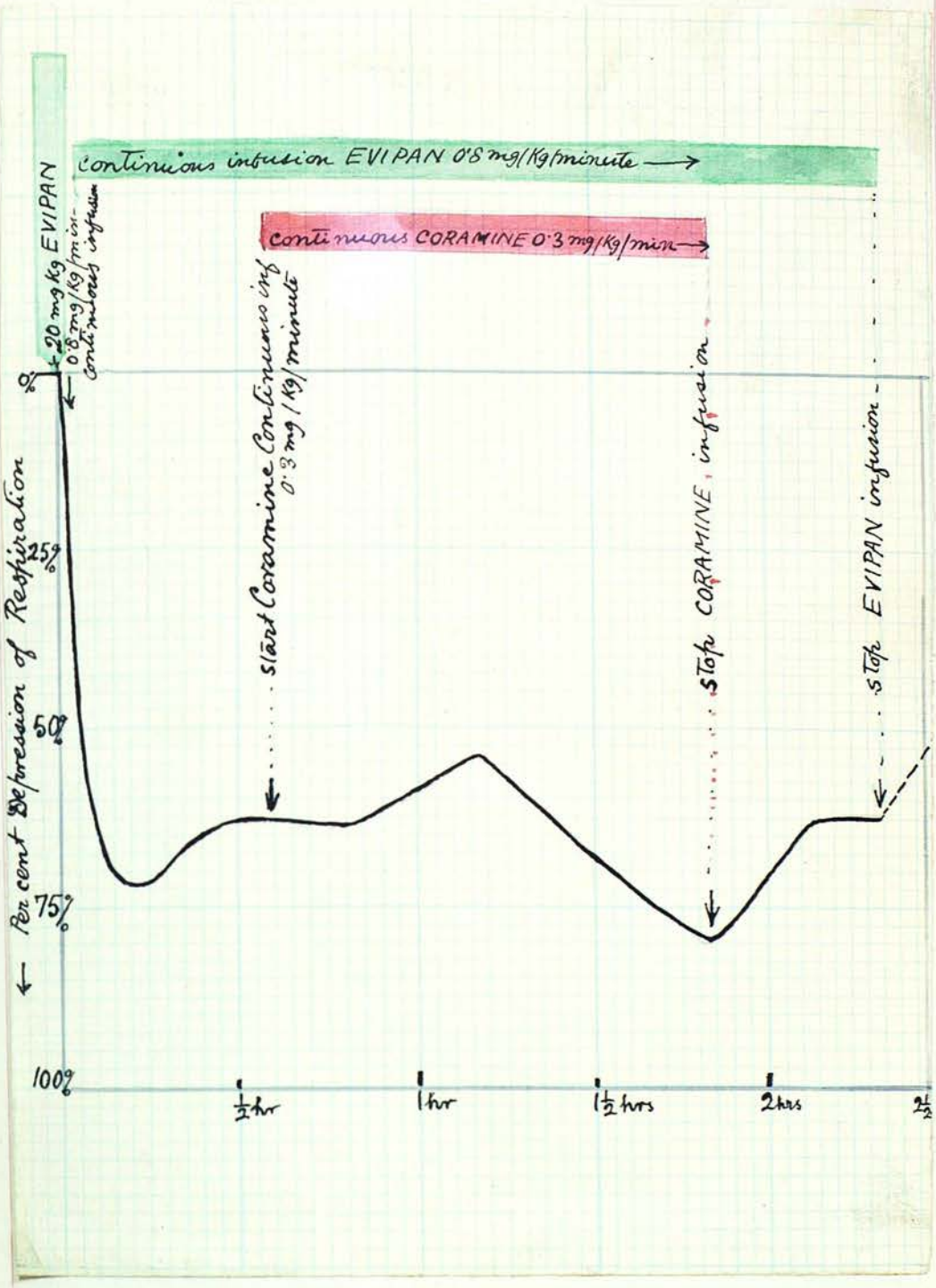


Fig. 30. Respiratory curve of another experiment showing the secondary depressant effect of continuous coramine infusion 0.3 mg./kg./min.

Discussion.

This biphasic action of continuous coramine infusion leads to the assumption that coramine before final disposal in the body is changed into an intermediary product of a depressant nature which is dealt with less quickly than coramine itself and can therefore accumulate in sufficient quantity to more than neutralise the stimulant action of coramine. Further, the depressant activity of this intermediary product seems to be more pronounced at greater depth of original depression. This explains why at deeper levels of depression proportionately lesser effect is obtained from coramine. Cases of death after coramine (much below toxic doses) can be explained by individual hypersensitiveness of the animals to this intermediary product of coramine metabolism.

This also explains the anomalous behaviour of coramine reported by some of the recent workers in the face of favourable reports of earlier literature. For example coramine in big doses was reported to have increased the depth or prolonged the duration of sleep caused by medinal (Tartler 1929), avertin (Jager, 1932; Lendle, 1936), Zipf et al. (1937); pernocton (Lendle, 1936/

1936, Zipf et al. 1937), veronal, luminal and somnefene (Moritsh, 1932), and evipan (Schwab and Jung, 1936; Zipf et al. 1937). Further, an increase of toxicity of the narcotic after coramine was reported by Moritsch (1932), Kohn and Jacobi (1935) and Zipf et al. (1937). Barlow (1935) mentions a secondary depression following coramine and Maloney (1935) speaks of coramine as contra-indicated in deep barbiturate depression for, "beyond a stage it adds to the depression and kills by depression and not by convulsion". Schwab and Jung (1936) suspected a "paralytic component of coramine". The results of the experiments with continuous coramine infusion suggest strongly that this suspected paralytic component is not present in coramine itself, but is, as suggested above, formed in the body in the shape of an intermediary product of coramine metabolism. The rapid breakdown of coramine may make it relatively safe or at any rate unlikely to cause convulsion. This advantage is, however, lost if coramine breakdown liberates some depressant agent.

Summary/

Summary.

1. Coramine was found to have a quick but short-lived action as a respiratory analeptic. It improved the rate of the respiration rather than the depth.

2. Its action was inconstant, and sometimes failed to respond.

3. It could not maintain the same equi-active dosage relationship with cardiazol and picrotoxin at deeper levels of depression that it showed at a higher level.

4. On a continuous infusion of coramine simultaneously with a continuous evipan, the respiratory curve at first showed a slow rise and then a gradual fall even below the previous evipan level, to which it returned after stopping the coramine infusion. This suggested the formation of an intermediary product during coramine metabolism, which had a depressant action and was more stable than coramine itself. The anomalous behaviour of coramine also reported by other workers could be explained in this way.

VII. CARDIAZOL AND STRYCHNINE.

The effects of single intravenous injections of cardiazol on depressed respiratory activity maintained at a steady level by continuous slow infusion of evipan, can be seen in Figs. 16(a), 27 and 28. Cardiazol produced a prompt response like coramine but the improvement was more marked as regards rate than depth, although in this latter respect, it was somewhat better than coramine. Furthermore, the action of cardiazol was found to be more constant than that of coramine when there was great depression of respiration. Cardiazol (10 mg./kg.) increased respiratory activity to the same extent as did 0.3 mg. of picrotoxin in the experiment represented in Fig.16(a)where the depression was at the 50 p.c. line, although the picrotoxin effect lasted longer. This equi-active relationship was maintained in experiment shown in Fig. 27 where depression was at about 60 p.c. as also in that shown in Fig. 28 where respiratory depression was at about 80 p.c. level.

The duration of action of various doses of cardiazol as obtained in different experiments is given in tabular form below. (Table VIII).

Dose/

Table VIII.

Dose of cardiazol per kg.	Log. of dose	Duration in min. as obtained in different expts.	Average duration in min.
5.0 mg.	0.69	5, 5	5
7.5	0.87	9	9
10.0	1.0	9,12,12,18,16,18	14
15.0	1.17	24, 18	21
20.0	1.3	24, 22, 24, 20	22.5
30.0	1.47	31, 29, 32	31
40.0	1.6	33, 35, 39	36
50.0	1.69	42, 40, 45	42

Plotting these doses with the average durations gives the curve shown in Fig. 25(a). If the logs. of these doses are plotted with the corresponding average duration, practically a straight line is obtained (Fig. 25(b)). This indicates that the duration of cardiazol action varies/

varies with the log. dose of cardiazol. The slope of this log. dose-duration line corresponds to half destruction (= decrease by log. 0.3) in 10 minutes or a loss of 1/13 per minute. Similar calculations with coramine showed a loss of 1/10 every minute.

The resuscitating power of cardiazol was seen in an experiment (Fig. 31) in which the respiration of a rabbit under continuous evipan (0.8 mg./kg./min.) stopped after 3 successive doses of 250 mg./kg. of cyclamide (morpholine nicotinate). On administration of 50 mg./kg. cardiazol together with artificial respiration, there was immediate resumption of respiratory activity which soon went up beyond 50 p.c. level.

Fig. 32 gives the results of an experiment in which cardiazol was continuously infused. As would be seen from the respiratory curve the improvement produced in respiratory activity could not be maintained by the drug although the subsequent fall in effect was much less marked than in the case of coramine.

Figs. 31 and 32/

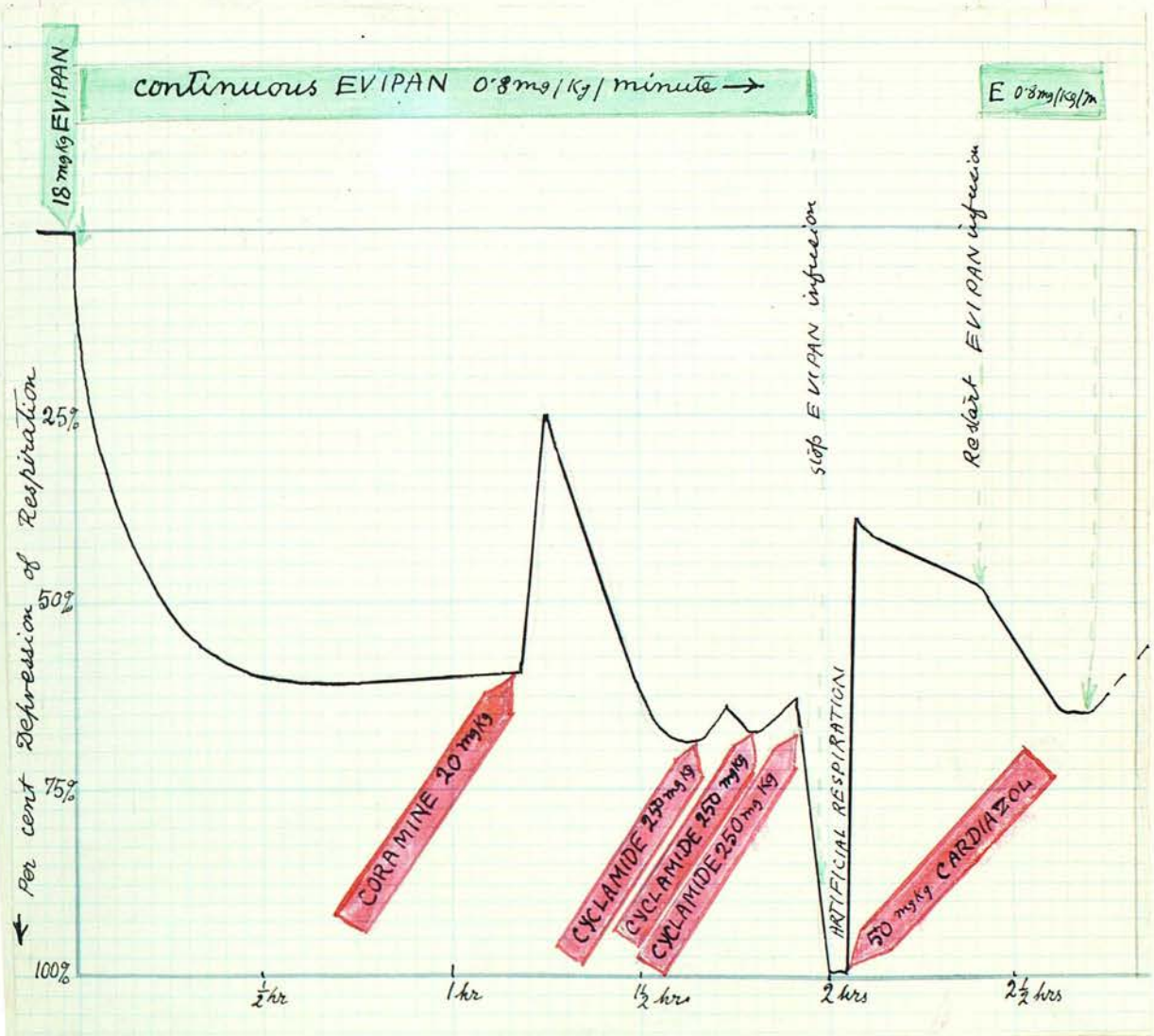


Fig. 31. Respiratory curve of an experiment in which basic depression was due to 0.8 mg./kg./min. of evipan after an initial dose of 18 mg./kg. of evipan. After 20 mg. of coramine, 3 successive doses of cyclamide 250 mg./kg. each, resulted in complete stoppage of respiration. Artificial respiration was started. 50 mg./kg. of cardiazol at once caused resumption of respiratory activity.

Fig. 32. Respiratory curve of an experiment showing the effect of continuous cardiazol infusion.

On gradually increasing the dose of the amount infused, from 0.4 mg./kg. to 0.5 mg./kg./min. this tendency became clearer and on continuing 0.5 mg./kg. for some time twitchings and slight convulsive tendency appeared and then regular convulsions although respiratory activity was at about 40 p.c. level.

Single injections of cardiazol at this level of depression do not give rise to convulsion unless the dose be big. It seems therefore that cardiazol also before destruction is converted into intermediary products, one of which is convulsive in nature. But besides this there has got to be some other factor which prevents continuous rise of respiratory activity and sometimes causes a diminution of the increased activity. In this respect it presents a more complicated picture than coramine.

Strychnine after a single injection gave improved respiration. 0.1 mg. of strychnine gave less effect than 0.3 mg. of picrotoxin (Fig. 7) although toxicity of strychnine for rabbit was found by Maloney to be ten times that of picrotoxin. 0.4 mg./kg. of strychnine gave rise to severe convulsion/

convulsion in an animal with 40 p.c. respiratory activity due to 0.75 mg./min. continuous evipan. Fig. 33 gives the result of continuous injections of strychnine. 0.006 mg. very soon and 0.002 mg. after some time gave rise to convulsion although respiratory activity was increased only to a slight degree by that time.

Fig. 33 /

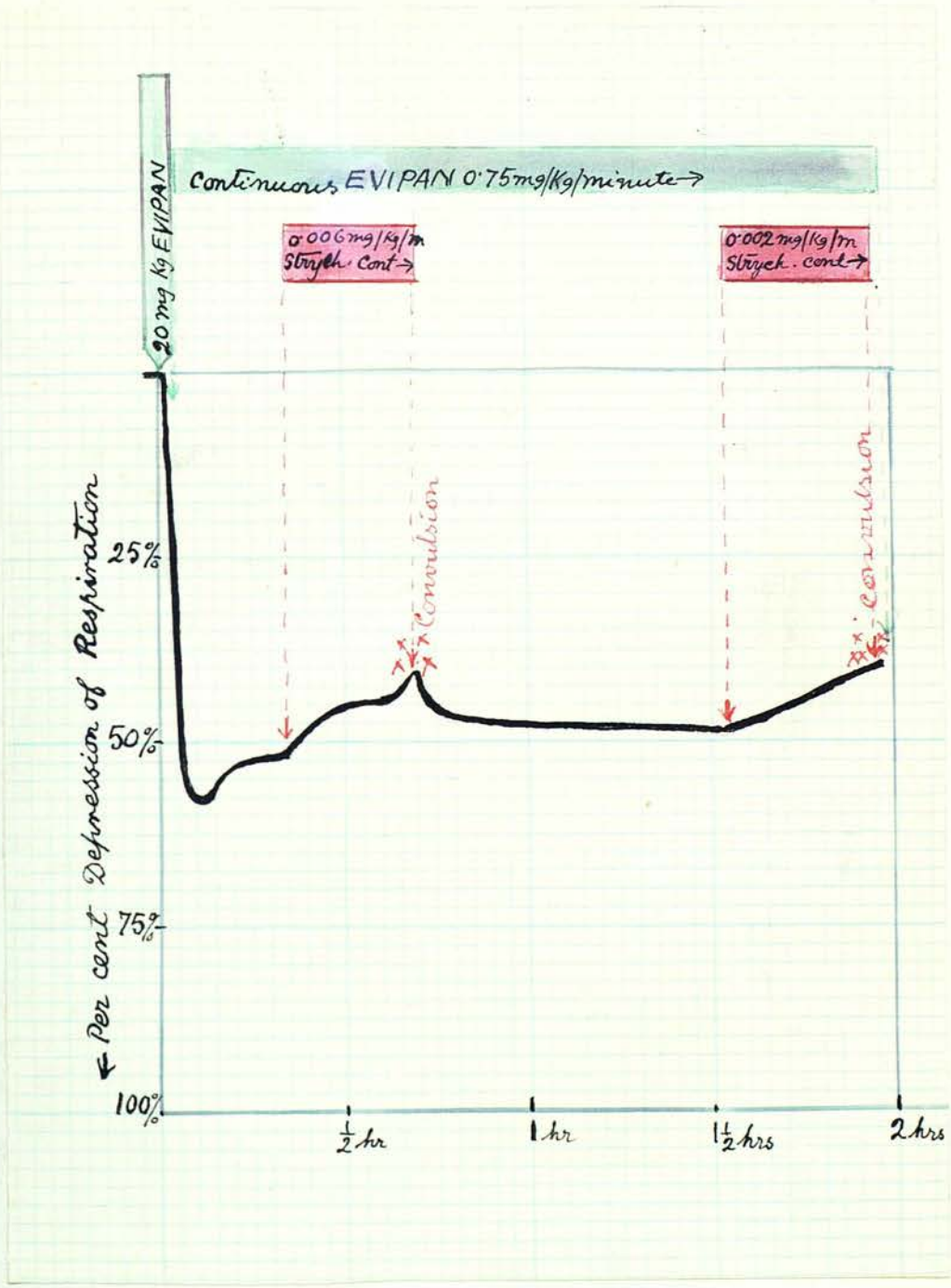


Fig. 33. Respiratory curve of an experiment showing the effect of continuous infusion of strychnine. Convulsions appeared although respiratory activity was about 40 per cent. below normal.

VIII. CIRCULATORY EFFECTS OF THE ANALEPTICS.

Earlier investigators stated that coramine was a stimulant to the circulation and that it acted on the myocardium and also produced vaso-constriction (Lagier, 1922; Faust, 1925; Asher, 1926). Uhlmann (1924), however, reported that moderate doses of coramine had hardly any stimulant effect on normal blood pressure. Massart (1930) found that 25 mg./kg. of coramine in dogs produced only a small rise of blood pressure. He further observed that after single massive doses of coramine there was,before the hypertensive response, a primary and transitory fall of blood pressure due to bradycardia of vagal origin. Killian and Uhlmann (1931) found this to be true for laboratory animals as well as for man. Van Esveld (1930) found that in decerebrate cats moderate doses of coramine had only a small effect on the blood pressure, while bigger doses caused convulsions. Stross (1928) found that in decapitated rabbits coramine failed to cause rise of blood pressure. Trendelenburg (1929) found that in vascular insufficiency and vasomotor paresis caused by novocaine, histamine and chloral hydrate, coramine/

coramine failed to improve the blood pressure. Issekutz (1935) reported that coramine failed to combat the circulatory depression caused by chloroform in cats.

Cardiazol has also been stated by various workers to cause a rise in blood pressure. Hildebrandt (1925) reported that in urethanised cats 2 to 14 mg./kg. of cardiazol caused a moderate rise of blood pressure of fairly long duration. There was an initial fall of pressure if the vagi were intact. Plethysmographic experiments indicated a vasoconstriction in the splenchnic area with a compensatory dilatation of blood vessels in the skin and muscles. Camp (1928) was unable to find any definite or constant action of cardiazol on the heart. A concentration of even 1 in 1500 did not produce any effect on the frog's heart. Contrary to Hildebrandt's findings, Camp showed by means of an oncometer, an increase of kidney volume after cardiazol. Goworow (1929) found that the ear vessels of the rabbit, when perfused with cardiazol, showed dilatation. Goworow and Speranskaja-Stepanowa (1931) analysed the action of/

of cardiazol on the blood pressure of cats and dogs. They found that the cardiazol effect depended on the tonus of the autonomic as well as of the central nervous system. Cardiazol caused a rise of blood pressure only when the tonus of the vaso-constrictor centre was intact. In paralytic conditions of the central nervous system, the vaso-constrictor centre was one of the earliest to suffer and in such conditions, cardiazol caused central vasodilatation and a fall of blood pressure.

The action of these drugs on the circulation is a matter of practical importance, because coramine and cardiazol are both very extensively employed in clinical practice as circulatory stimulants. The author therefore made experiments to compare the circulatory effects of various analeptic drugs.

In one experiment the blood pressure of a rabbit had fallen to about 15 mm. Hg in consequence of prolonged manipulation and exposure of the abdominal viscera, but neither 30 mg./kg. of coramine nor 20 mg./kg. of cardiazol produced any improvement. Observations on many cats showed that/

that towards the end of lengthy experiments, when the blood pressure had fallen to a low level, these drugs often failed to produce any improvement. This confirmed Trendelenburg's (1929) finding as regards coramine and that of Gorowow and co-workers (1931) as regards cardiazol.

It was found that with rabbits 10 mg./kg. of coramine, 10 mg./kg. of cardiazol and 0.3 mg./kg. of picrotoxin were equi-active doses so far as respiratory analeptic activity was concerned. The vasomotor effects of these drugs in the doses were therefore compared in cats under chloralose anaesthesia. Both coramine and cardiazol had a prompt effect in causing a rise of blood pressure, but the effect was maintained by cardiazol for nearly twice as much time as coramine. As regards intensity of action also cardiazol was about twice as active as coramine.

There was a marked difference between the effects of these drugs and that of picrotoxin. As in the case of its respiratory stimulant action, the circulatory effects of picrotoxin developed slowly but lasted longer (twice as long as the action of cardiazol). The extent of the rise of blood/

blood pressure was nearly the same as that produced by cardiazol.

In other experiments the behaviour of these drugs was studied on the lowered blood pressure caused by injection of evipan. A dose of 10 mg./kg. of evipan was given to a cat under chloralose anaesthesia. A typical sharp fall of blood pressure and a slow rise back to normal was obtained (Fig. 34). Some time after, the same dose of evipan was again given, and when the maximum depression was produced, a dose of 15 mg./kg. of coramine was injected. This caused an improvement but did not fully counterbalance the evipan effect. Moreover the intensity of the coramine effect passed off quickly and so the evipan effect was manifest again. After an interval the same procedure was repeated with 10 mg./kg. of evipan and 10 mg./kg. of cardiazol. The evipan effect was practically counterbalanced by the cardiazol, but the peak of cardiazol effect passed off quickly and the evipan depression re-appeared to some extent. After another interval, the same procedure was again repeated with

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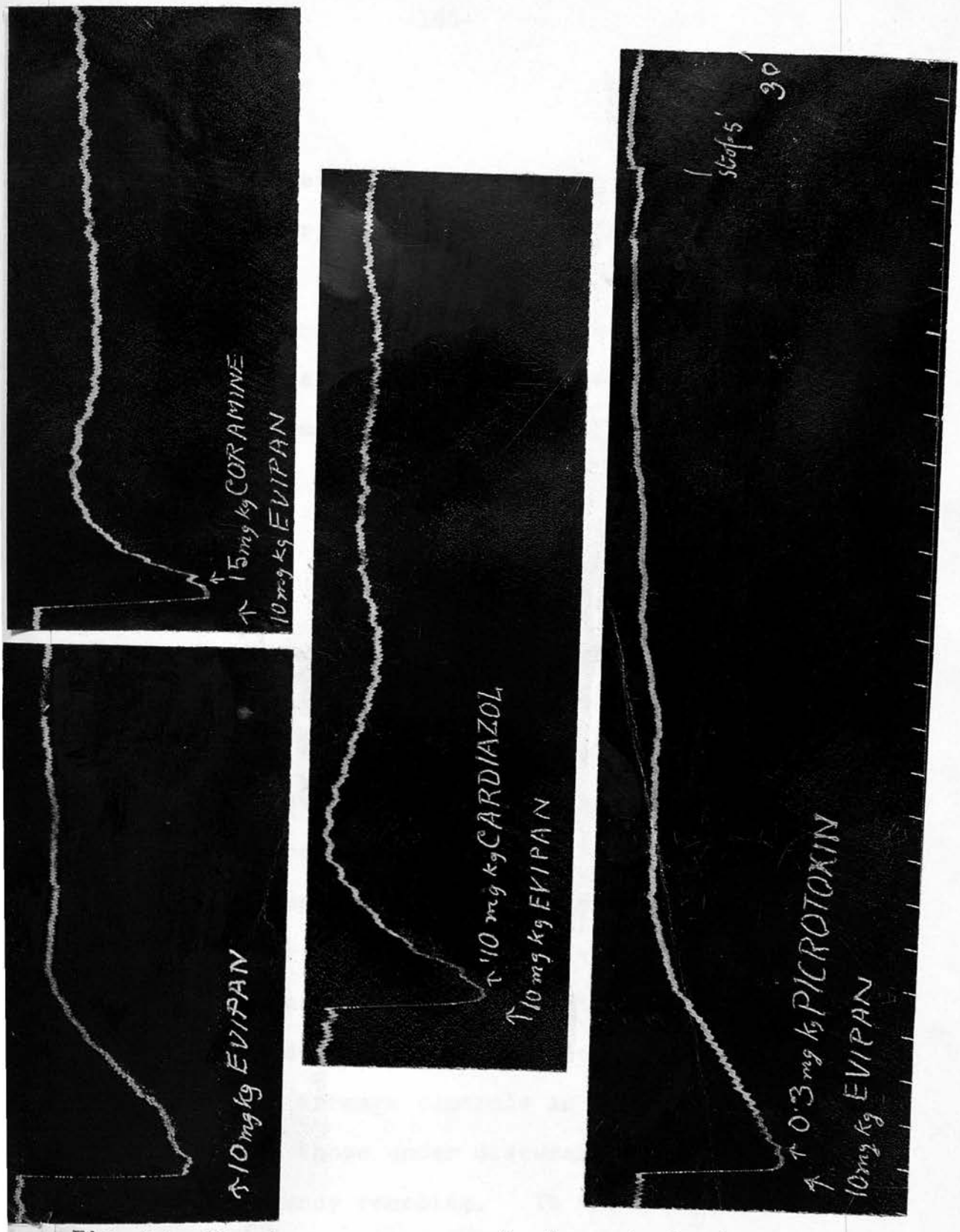


Fig. 34. Blood pressure record of a cat showing effects of the analeptics on the blood pressure depressed by evipan. Details in the text.

10 mg./kg. of evipan and 0.3 mg./kg. of picrotoxin. There was a very slight effect on the blood pressure curve in the beginning, but the blood pressure continued to rise steadily to slightly above the normal level, and this effect was sustained for more than 30 minutes.

Discussion.

Favourable reports about coramine and cardiazol as circulatory stimulants come mostly from clinical workers. But the majority of experimental evidence points to their questionable utility as circulatory stimulants in grave circulatory depression, especially when this accompanies or follows the depression of the central nervous system. The clinical evidence must be regarded as uncertain, because it is impossible to arrange controls in the case of drugs such as those under discussion which are used as emergency remedies. It seems probable that the favourable results of clinical work have been due to the use of these drugs in cases where the/

the vasomotor centre was not severely depressed.

It has been seen that after barbiturates there is a fall of blood pressure. In the case of evipan this is due to vasodilatation of central as well as of peripheral origin and also to cardiac depression. Cases of barbiturate depression that call for the use of an analeptic are cases in which the respiratory centre has been severely depressed. Such a depression of the respiratory centre would be usually accompanied by circulatory depression, caused by loss of tonus of the vasoconstrictor centre. Apart from whatever respiratory analeptic action coramine and cardiazol may exert in such cases, the circulatory depression is not likely to be combatted by them in absence of tonus of the vasomotor centre. Moreover their action is so short-lived that they cannot exert a prolonged beneficial effect on the circulation. Picrotoxin has a prolonged period of activity and would have proved a very useful agent against circulatory depression in such cases but unfortunately the action of this drug is slow to develop. The best way to combat such circulatory depression appears to be a

combination/

combination of a sympathomimetic drug and picrotoxin. The first will initiate the circulatory improvement and the second will keep up the improvement for a long time.

Summary.

1. In cases of severe vasomotor depression coramine and cardiazol fail to produce circulatory improvement.

2. Coramine and cardiazol produce prompt circulatory improvement in cases where the depression is not severe. Cardiazol has a circulatory action of longer duration than, and double the intensity of, a dose of coramine of equal respiratory analeptic value.

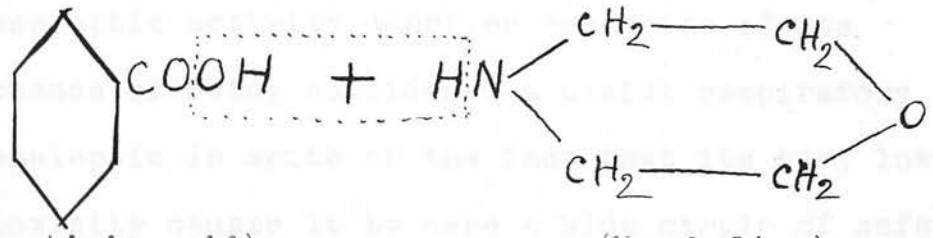
3. Picrotoxin has a very long period of activity as a circulatory stimulant and the intensity of its action is nearly equal to that of cardiazol, but its action is very slow to develop.

IX. TESTING OF NEW COMPOUNDS FOR ANALEPTIC EFFECTS.

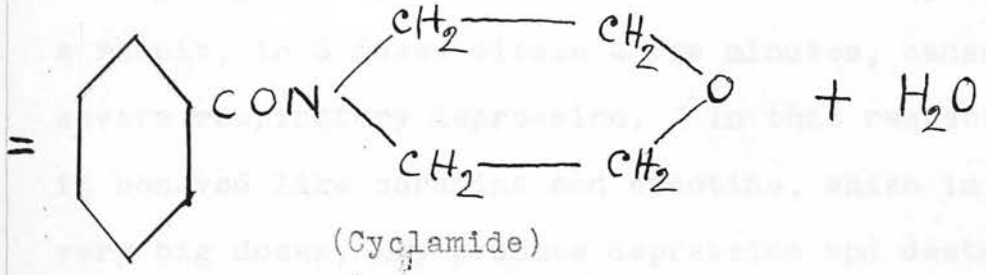
The method employed for the study of the analeptic effects of picrotoxin, coramine, cardiazol and strychnine, also proved to be a very efficient method for testing of new compounds for evidence of analeptic activity. The method (described in detail in a previous chapter) essentially consisted in the production of a steady condition of respiratory depression of a rabbit by a continuous infusion of sodium evipan (one mg./kg./min. with a suitable initial dose). The drug to be tested was then injected intravenously in various doses and the effects of the drug on respiration could be read off from the respiratory curve. In cases where there was evidence of respiratory stimulation the effects produced were compared with those produced by a known analeptic (coramine or picrotoxin), both in respect of the intensity of stimulation and the duration of action. A series of new compounds were tested in this way. The following description of the testing of cyclamide will serve as an illustration.

Cyclamide/

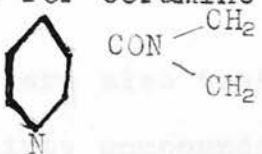
Cyclamide or morpholine nicotinate is a compound formed by adding morpholine radicle to nicotinic acid with elimination of H_2O.



(Nicotinic acid) (Morpholine)



(Cyclamide)

It was intended as a substitute for coramine (nicotinic acid diethylamide ).

Cyclamide was found to be much less toxic than coramine. Even 5 gram per kg. of cyclamide did not kill frogs, while frogs of the same batch and under similar conditions were killed by less than 1 gram per kg. of coramine. 300 mg./kg. of coramine was found to be the median lethal dose for mice whereas even 2500 mg./kg. of cyclamide did not cause death of any of the mice.

The analeptic activity of cyclamide, however, was found to be very low. A dose of

100/

100 mg./kg. of cyclamide produced less intense effect than 25 mg./kg. of coramine although the duration of action was much longer. This low analeptic activity deprives cyclamide of its chance of being considered a useful respiratory analeptic in spite of the fact that its very low toxicity causes it to have a wide margin of safety. 750 mg./kg. of cyclamide, given intravenously to a rabbit, in 3 doses within a few minutes, caused severe respiratory depression. In this respect it behaved like coramine and nicotine, which in very big doses, may produce depression and death without causing convulsion.

The other new compounds were also tested in the same way as cyclamide. Thirty compounds were tested; five of them proved to be respiratory depressants; five showed no appreciable action even with a dose of 100 mg./kg.; and sixteen showed a very slight analeptic activity. Only four compounds showed moderate analeptic action and their activities varied from 20 p.c. to 75 p.c. of that of coramine. They did not therefore possess any claim to come into the field of practical therapeutics in preference to the already established analeptics.

X. CONCLUSIONS.

The continuous intravenous infusion of sodium evipan produces a respiratory depression that remains constant in its intensity for hours. The results indicate that a constant fraction (namely 1/25) of the evipan present in the body is destroyed per minute.

The steady state of respiratory depression produced in the above way provides a very efficient method for the quantitative study of the analeptic activity of drugs. Single injections of analeptics superimposed on the continuous infusion of evipan give information about their intensity as well as the duration of their action. The former provides the basis for comparison of different analeptics and the latter gives data for calculating the rate of detoxication of the analeptics. The simultaneous continuous infusion of evipan and an analeptic also yields interesting results. For example, different doses of continuous evipan can be antagonised by suitable doses of continuous picrotoxin and these doses of evipan vary with the logarithm of the dose of picrotoxin required.

The/

The results of the study of the analeptic drugs dealt with in the foregoing chapters, show that picrotoxin is much more effective than either coramine or cardiazol in antagonising the depressant action of sodium evipan and are in general agreement with the findings of previous workers with respect to other barbiturates. This superiority of picrotoxin is shown most clearly in cases of great respiratory depression. Picrotoxin will produce a stimulant respiratory effect even after the respiration has been arrested by evipan, whereas coramine often fails to stimulate respiration when this is depressed to about one fifth of the normal. Moreover in such cases it may even augment the depression. Cardiazol is intermediate between picrotoxin and coramine.

Experiments with continuous infusion of coramine simultaneously with continuous evipan have shown that coramine, after an initial period of stimulation, leads to depression which is greater than what would be due to evipan alone. This strongly suggests that coramine is broken down to form some more stable intermediary product which has a depressant action. The rapid breakdown of coramine/

coramine may make it relatively safe or at any rate unlikely to cause convulsion. This advantage is however lost if coramine breakdown liberates some depressant agent.

The chief properties of these analeptics are summarised in Table IX.

Table IX.

Drug	Minimum effective dose mg./kg. (M.E.D.)	Minimum convulsive dose mg./kg. (M.C.D.)	Safety ratio i.e. M.C.D./M.E.D.	Duration of action of double the min. effective dose in minutes
Picrotoxin	0.2	0.8	4	30
Coramine	3	60	20	6
Cardiazol	3	45	15	8

Picrotoxin has the most powerful action and the action is of relatively long duration. The obvious disadvantage of this drug is that there is a relatively narrow margin between the minimum effective and convulsive doses. This disadvantage is, however, lessened by the fact that barbiturates antagonise/

antagonise the convulsive action of picrotoxin.

The outstanding characteristics of coramine and cardiazol are that they have an action of short duration and a wide margin of safety, and their stimulant action is produced very rapidly. The clinical popularity of these drugs is probably due to these properties. My experiments show clearly, however, that of the three drugs, picrotoxin alone is likely to be effective in severe respiratory failure produced by overdose of sodium evipan.

The writer gratefully acknowledges his indebtedness to Professor A.J. Clark for his guidance and advice throughout the course of this work.

The expenses for the animals of this research were defrayed by a grant from the Moray Research Fund of the University of Edinburgh, for which the writer expresses his thanks.

The appendix comprises copies of 2 papers that are awaiting publication.

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ANTAGONISM OF EVIPAN BY PICROTOXIN, CORAMINE, AND CARDIAZOL. By S. C. DAS. From the Department of Pharmacology, University of Edinburgh.

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ANTAGONISM OF EVIPAN BY PICROTOXIN, CORAMINE, AND CARDIAZOL.

By S. C. DAS. From the Department of Pharmacology, University of Edinburgh.

(*Received for publication 6th July* 1939.)

THE antagonism between barbiturates and analeptics has been investigated in different ways by various authors. Maloney and co-workers [1931, 1932, 1933] gave graded doses of barbiturates to rabbits and followed these by varying doses of picrotoxin. They took the life-saving capacity of the latter drug as the chief measure of its analeptic efficiency. Koppanyi *et al.* [1936] produced hypnosis in rabbits by varying doses of nembutal, and measured the activity of picrotoxin by its power to cause awakening. This antagonism is presumably due to antagonistic actions of the drugs on the central nervous system. Another possibility is that analeptics increase the rate of detoxication of barbiturates. The latter explanation is improbable, and in the case of the antagonism of avertin by analeptics Beck and Lendle [1932] showed that in rabbits the rate of detoxication of avertin was not affected by analeptics given in doses sufficient to antagonise the hypnotic.

The chief purpose for which analeptics are used clinically is the stimulation of the respiratory centre, and the most promising method of investigating the action of analeptics is to measure their effects on the respiratory centre after this has been depressed. The chief difficulty is to obtain a constant degree of depression of the respiratory centre. One method of producing this effect is to administer some drug such as phenobarbitone, which has a very prolonged action. The defensive mechanism of the body against drugs is, however, so complex and efficient that it is difficult to obtain a prolonged and uniform depression by such means. An alternative method is to give a continuous intravenous infusion of some drug which is quickly destroyed.

The author showed [1939] that a steady state of respiratory depression in rabbits can be produced and maintained for hours by giving an initial intravenous dose of 15–20 mg./kg. of sodium evipan and then maintaining a continuous intravenous infusion, at a rate of about 1 mg./kg./min. of evipan.

The uniformity of the infusion was ensured by the use of an apparatus

that maintained a steady flow of 0·1 c.c. per minute. Respiratory activity was recorded by Cushny's method [1913]. The animal was covered with a plethysmograph which was connected to a float recorder. The respiratory activity was measured by multiplying the frequency, and the amplitude of the respiratory movements recorded on the smoked drum by the writing-point of the recorder. The results were expressed as percentage of the normal respiration activity, and by plotting these values against time, curves of respiratory activity for the whole period of the experiment were obtained.

A. Picrotoxin.

Two types of experiments were carried out:

(1) Continuous intravenous evipan was given at a rate which produced a steady respiratory depression. Single doses of picrotoxin were then injected and the amplitude and duration of the respiratory stimulation were measured. The same method was employed to compare the action of picrotoxin with those of other analeptics.

(2) Measurements were made of the antagonism between a continuous infusion of evipan and a continuous infusion of picrotoxin. This method permitted the balancing of evipan and picrotoxin, and it was possible to work up to a combination of doses of the two drugs, either of which alone would have been fatal.

1. *Antagonism of Evipan by Single Doses of Picrotoxin.*

When a rabbit whose respiration was maintained in a steady state of depression by a continuous infusion of evipan received a dose of 0·3 mg./kg. of picrotoxin, there was a temporary stimulation of respiration lasting for 20–30 min. The improvement was both in respect of the depth of respiration as well as the rate, and it took about 5 min. to reach the height of improvement. The temporary nature of the effect indicates that picrotoxin is fairly rapidly detoxicated in the body. Since a dose of 0·2 mg./kg. produced no certain stimulation of respiration, the result indicates that picrotoxin is reduced to 2/3 in about 30 min. If the destruction follows an exponential course, then the loss corresponds to a destruction of about 1·4 per cent. per minute. A second dose of picrotoxin, given about an hour after the first, produced an identical response, a fact which supports the view that the picrotoxin is completely detoxicated in the body in a fairly short time. When the effect of one dose had passed off, the respiratory depression reached the previous steady level on account of the continuous infusion of evipan, and a dose of the same or of another drug could now be given. By successive injections into the same rabbit, different analeptics were quantitatively compared under similar conditions. In general, 0·3 mg. of picrotoxin produced as much increase of respiratory

activity as did 10 mg. of coramine or 10 mg. of cardiazol, but the duration of action of picrotoxin was about double that of the latter drugs. Fig. 1 illustrates one such experiment. A remarkable feature

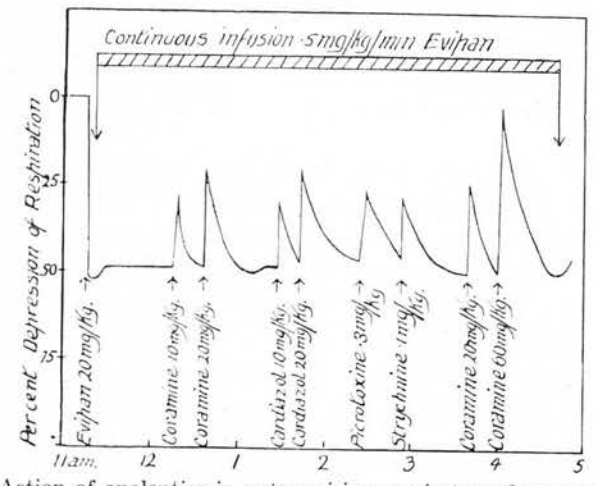

Fig. 1.—Action of analeptics in antagonising respiratory depression produced by sodium evipan in rabbits. Ordinate: percentage of respiratory depression; abscissa: time in hours.

of this figure is the steady basic level of respiratory depression which was maintained for 5 hours.

2. *Antagonism between Continuous Intravenous Infusion of Evipan and of Picrotoxin.*

In an experiment of this type, 0·6 mg./kg./min. of evipan, continuously infused, maintained a steady depression of respiration for about an hour. Then 0·3 mg./kg. of picrotoxin was administered, and it caused a partial recovery which was maintained at a constant level by a continuous infusion of 0·004 mg./kg. of picrotoxin per minute. This result indicates that with this rate of infusion, the rate of destruction of picrotoxin in the body nearly balanced the rate of infusion. This indicates a destruction of 0·004/0·3 or 1/75 per minute of the drug present.

More striking results were obtained when infusions of evipan were given which would have caused respiratory arrest if not antagonised by picrotoxin. In one experiment 1·2 mg./kg./min. of continuous evipan (following an initial dose of 15 mg./kg. of evipan) produced a progressively increasing depression, and a dose of 0·7 mg./kg. of picrotoxin followed by 0·008 mg./kg./min. produced a steady level of partial recovery. This indicates a destruction per minute of 0·008/0·7 = 1/87 of the picrotoxin present.

In another experiment, the partial recovery produced by 0·9 mg./kg. of picrotoxin could not be maintained by 0·006 mg./kg./min. of picro-

toxin continuously infused. Hence the amount destroyed was more than 0·006/0·9 or 1/150 per minute. In another case of progressive depression caused by 2 mg./kg. per min. of evipan, partial recovery caused by 0·7 mg./kg. of picrotoxin could be maintained by 0·015 mg./kg./min. of continuous picrotoxin. The figures suggest that there is a considerable individual variation, and that the amount of picrotoxin removed per minute varies between 1 and 2 per cent. of the amount present.

The results obtained with continuous simultaneous infusion of picrotoxin and of evipan are summarised in Table I. If the logs. of picrotoxin doses are plotted against the corresponding evipan doses, a linear relationship is obtained, as seen in fig. 2. The nature of these

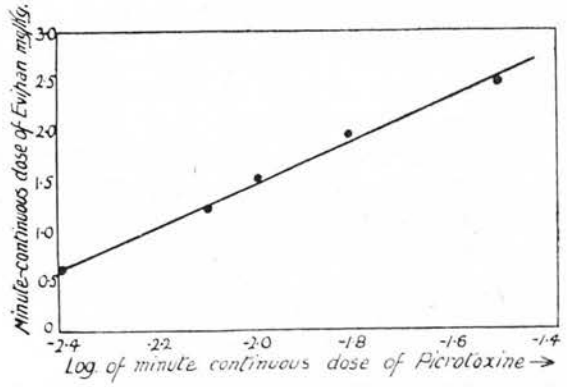

Fig. 2.—Relation between the rates of simultaneous infusion of sodium evipan and of picrotoxin which maintain steady respiratory depression in rabbits. Dose of sodium evipan plotted against log. of dose of picrotoxin.

results agrees in general with those obtained by Maloney and co-workers [1931], and also by Koppanyi *et al.* [1936]. This relation explains why it is possible to antagonise a wide range of picrotoxin dosage by barbiturates, whilst only a comparatively narrow range of barbiturate dosage can be antagonised by picrotoxin.

Interpretation.—The results of the experiments with continuous infusion of evipan alone have, as reported in a previous communication [Das and Raventós, 1939], led to the assumption that in rabbits about 1/25 (or 4 per cent.) of the evipan present is destroyed per minute. In the case of picrotoxin a single dose of 0·3 mg./kg. acts for about 20 minutes. The minimum effective dose was found to be (*a*) 0·2 mg./kg. in the case of single injections, and (*b*) 0·003 mg./kg./min. in the case of continuous infusions. A destruction rate of 1/70 per minute would account for this ratio. The estimates of the rate of picrotoxin destruction vary from 1/48 to 1/87 per minute, or between 1 and 2 per cent. per minute of the picrotoxin present. This rate is considerably slower than the rate found for evipan (3 to 5 per cent. per minute).

Table I.—Results Obtained with Continuous Simultaneous Infusion of Picrotoxin and Evipan.

Per min. dose of continuous evipan, mg.	Per min. dose of continuous picrotoxin, mg.	Duration of simultaneous infusion (in hours).	Results.	Equivalent doses. E = evipan. P = picrotoxin.	
0·6	0·004	2	Antagonised.	0·6 E	0·004 P
1·2	0·008	2	Antagonised.	1·2 E	0·008 P
1·5	0·006	¾	Picrotoxin too little to antagonise; 0·01 would probably antagonise.	1·5 E	0·01 P
2·0	0·015	1½	Antagonised.	2·0 E	0·015 P
2·5	0·03	1½	Antagonised.	2·5 E	0·03 P

In the case of evipan it was possible to produce fairly conclusive proof that a fixed proportion of the drug present was removed per minute. The evidence in the case of picrotoxin is less conclusive, but Table I. shows that it is possible to maintain a steady level of partial recovery of respiratory activity for hours, and it is difficult to imagine how such a result could be obtained unless a constant portion of the drug present is destroyed per minute, since only this mechanism will result in a constant intravenous infusion producing a steady effect.

B. Coramine and Cardiazol.

Coramine was studied in the same manner as has been described in the case of picrotoxin. A steady depression of respiration was produced in the rabbit by continuous intravenous infusion of evipan, and the stimulant action of coramine on respiration was estimated firstly by single injections, and secondly by continuous infusion.

1. (a) (i) *Effect of Single Doses of Coramine.*

Coramine injected intravenously into rabbits whose respiration was in a steady state of depression on account of continuous evipan caused a prompt rise in respiratory activity, mostly due to an increase in the respiration rate rather than the amplitude of respiration. The quick rise was followed by a sharp fall of the respiratory activity. There was slight variation in the duration of action of the same dose in different cases. The average of the figures obtained in different experiments is given in Table II.

TABLE II.

Dose per kg. of coramine in mg.	5	10	20	40	60
Log. of the dose . . .	0·69	1·00	1·30	1·60	1·77
Average duration in min. .	5	11·5	18	23	28

If the logs of these doses are plotted with the corresponding average duration a straight line is obtained (fig. 3 (CRM)). This indicates that

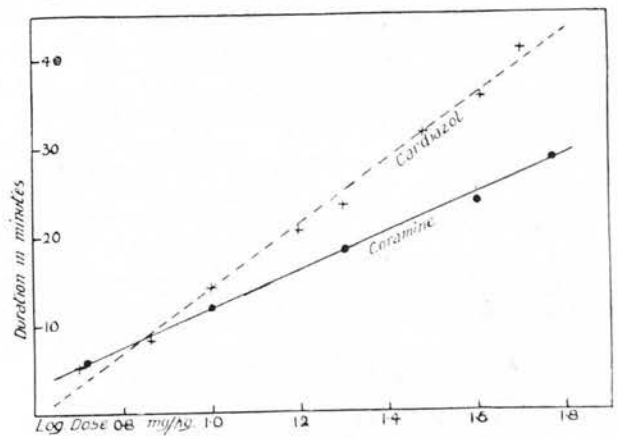

FIG. 3.—Log. dose-duration relation of action of cardiazol and of coramine on rabbit's respiration.

the duration of coramine action varies with the log. dose of coramine. Extrapolation of the curves indicates that 3 mg./kg. would produce no action. The slope of the curve, when log. dose is plotted against time, corresponds to half-destruction (decrease by log. 0·3) in about 7 minutes, or a loss of 1/10 per minute.

A continuous infusion of 0·25 mg./kg./min. produced an appreciable effect. This should produce a maximum accumulation of 2·5 mg./kg. This is just below the minimum effective dose.

(ii) *Single Doses of Cardiazol.*

Single doses of cardiazol gave a prompt response like coramine. The improvement was more marked as regards the rate than the depth of respiration, although in this latter respect cardiazol was somewhat better than coramine. The average duration of different doses of cardiazol computed from a number of experiments is given in Table III. The average duration is plotted with the log. of the corresponding dose in fig. 3 (curve CZL). As in the case of coramine, the log. dose-duration relationship is a straight line. The slope of this line indicates that a

difference of log. 0·3 (=time of half-destruction) occurs in 11 minutes, which corresponds to a detoxication at the rate of 1/16 per minute of the amount present.

TABLE III.—AVERAGE DURATION OF DIFFERENT DOSES OF CARDIAZOL COMPUTED FROM A NUMBER OF EXPERIMENTS.

Dose in mg. per kg. of cardiazol.	5	7·5	10	15	20	30	40	50
Log. of the dose .	0·69	0·87	1·0	1·17	1·3	1·47	1·6	1·69
Average duration in min.	5	9	14	21	22·5	31	36	42

(b) *Comparison of Single-dose Effects of the Analeptics.*

As already stated in connection with picrotoxin, the intensity of action of 10 mg. of coramine was in general equal to that of 10 mg. of cardiazol or of 0·3 mg. of picrotoxin. But it was noticed that coramine did not always keep up this equiactive dosage relationship. This was especially so after severe depression had been produced by evipan. In one experiment, for example, where the basic level of depression due to evipan infusion was 80 per cent. below normal, the effect of even 20 mg./kg. coramine was less than that of 10 mg. of cardiazol or of 0·3 mg. of picrotoxin. In another case where the respiratory depression was about 95 per cent., 30 mg. of coramine failed to produce any effect, whereas 0·3 mg./kg. of picrotoxin produced an improvement, whilst 0·4 mg./kg. of picrotoxin given shortly afterwards restored the respiration nearly to normal. In other instances also, where depression was rapidly increasing, large doses of coramine failed to stimulate, and in some cases actually caused depression.

2. (i) *Continuous Infusion of Coramine.*

In this group of experiments, after obtaining a steady level of depression with a continuous infusion of evipan, coramine was also simultaneously given by continuous infusion. With a dose of 0·2 mg./kg. of coramine per minute, no change was observed in the amount of respiratory activity. A dose of 0·25 mg./kg./min. caused a very slow increase in the respiratory activity, but on continuing the coramine infusion for some time it was noticed that not only the increased respiratory activity was not maintained, but there was a progressive decrease in the respiratory activity even below the basic level of evipan depression. Stopping the coramine at this stage, but continuing the evipan infusion, actually led to increased respiratory activity till the basic level of evipan depression was reached. With a continuous

infusion of 0·3 mg./kg./min. of coramine a still clearer picture was obtained. Fig. 4 illustrates the result of one such experiment.

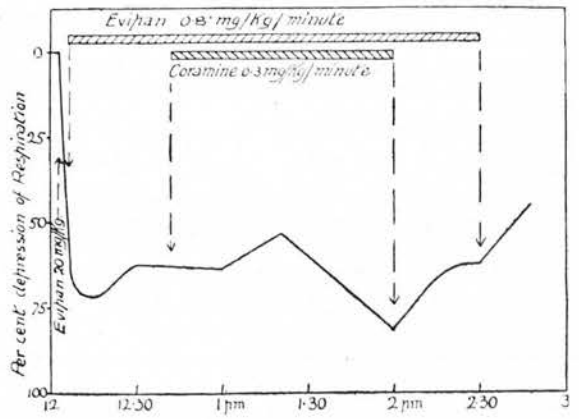

FIG. 4.—Effect of coramine on respiratory activity of rabbit when this is greatly depressed by sodium evipan. Ordinate: percentage of respiratory depression; abscissa: time in hours.

(ii) *Continuous Infusion of Cardiazol.*

With a continuous infusion of cardiazol superimposed on a continuous infusion of evipan, no effect was produced with doses below 0·35 mg./kg. per minute of cardiazol. A dose of 0·4 mg./kg./min. produced a small improvement with a subsequent fall in activity, though in this respect this was much less marked than coramine. On gradually increasing the dose from 0·4 to 0·5 mg./kg./min. the above tendency became clearer, and on continuing 0·5 mg. for some time, twitchings and slight convulsive tendency appeared and then regular convulsions came on, although the respiratory activity was in general about 35 per cent. below normal. Single injections of cardiazol at this level of respiratory depression do not give rise to convulsions unless the dose be fairly large. It may be that one component of the breakdown product of cardiazol can produce convulsions and at the same time counteracts the respiratory stimulant action of cardiazol, which therefore cannot keep up the respiratory stimulation. A more complicated picture is presented here than in the case of coramine.

DISCUSSION.

My results confirm those obtained by previous workers, and show that picrotoxin is much more effective than either coramine or cardiazol in antagonising the depressant action of sodium evipan on the respiratory centre. This superiority is shown most clearly in cases of great respiratory depression. Picrotoxin will produce a stimulant respiratory effect even after the respiration has been arrested by evipan, whereas coramine

often fails to stimulate respiration when this is depressed to about 1/5 of the normal. Moreover, in such cases it may even augment the depression. Cardiazol is intermediate between picrotoxin and coramine.

Continuous infusion with coramine superimposed on continuous infusion with sodium evipan usually produces an initial respiratory stimulation, but within about half an hour the stimulation passes off, and is followed by a depression which is greater than that produced by sodium evipan alone. This effect, together with the depressant effect produced by large doses of coramine, suggests that the coramine is broken down to form some more stable product which has a depressant action. This also explains the anomalous behaviour of coramine reported by some of the recent workers in the face of favourable reports of earlier literature. For example, coramine in large doses was reported to have increased the depth or prolonged the duration of sleep caused by medinal [Tartler, 1929], avertin [Jager, 1932; Lendle, 1936; Zief *et al.*, 1937], pernocton [Lendle, 1936; Zief *et al.*, 1937], veronal, luminal and somnefene [Moritsch, 1932], and evipan [Schwab and Jung, 1936; Zief *et al.*, 1937]. Further, an increase of toxicity of the narcotic after coramine was reported by Moritsch [1932], Kohn and Jacobi [1935], and Zief *et al.* [1937]. Barlow [1935] mentioned a secondary depression following coramine, and Maloney [1935] reported that beyond a stage coramine added to depression and killed by depression and not by convulsion. Schwab and Jung [1936] suspected a "paralytic component of coramine." The results of the experiments with continuous coramine infusion suggest strongly that this suspected paralytic component is not present in coramine itself but is, as suggested before, formed in the body as an intermediary product of coramine metabolism. The rapid breakdown of coramine may make it relatively safe, or at any rate unlikely to cause convulsions. This advantage is, however, lost if coramine breakdown liberates some depressant agent.

The chief properties of the three analeptics studied are summarised in Table IV. Picrotoxin has the most powerful action, and the action

TABLE IV.

	Minimum effective dose (M.E.D.), mg./kg.	Minimum convulsive dose (M.C.D.), mg./kg.	Safety ratio, *i.e.* M.C.D./ M.E.D.	Duration of action of double the minimum effective dose (in min.)	Time in min. for half-destruction.
Picrotoxin .	0·2	0·8	4	30	46
Coramine .	3	60	20	6	10
Cardiazol .	3	45	15	8	11

is of relatively long duration. The obvious disadvantage of this drug is that there is a relatively narrow margin between the minimum effective and convulsive doses. This disadvantage is, however, lessened by the fact that barbiturates antagonise the convulsive action of picrotoxin.

The outstanding characteristics of coramine and cardiazol are that they have an action of short duration and a wide margin of safety, and their stimulant action is produced very rapidly. The clinical popularity of these drugs is probably due to these properties. My experiments show clearly, however, that of the three drugs, picrotoxin alone is likely to be effective in the case of severe respiratory failure produced by overdose of sodium evipan.

It is important to note that these conclusions only apply to the antagonism of the depression produced by barbiturates. Results recorded in the literature show clearly that the relative efficiency of the drugs is different as regards other respiratory depressants, such as morphine. I have confirmed this conclusion in some preliminary experiments.

Summary.

1. The production of a steady state of respiratory depression by continuous intravenous infusion of sodium evipan into rabbits affords a convenient method for measuring the efficiency of analeptic drugs and for comparing their relative activities.

2. Picrotoxin causes an increase in the depth of respiratory movements as well as in the rate, whereas coramine and cardiazol improve the rate of respiration rather than the depth.

3. 0·3 mg. of picrotoxin causes the same increase in amplitude of respiratory stimulation as does 10 mg. of coramine or of cardiazol. Picrotoxin takes about 3–5 minutes to develop its maximum effect, whilst the action of coramine or of cardiazol reaches its maximum very quickly.

4. When the respiratory depression is severe, coramine often fails to produce a stimulant effect and may even augment the depression. Picrotoxin still produces a stimulant effect in such cases.

5. Picrotoxin is detoxicated at the rate of 1/70 of the amount present per minute, whereas the rate of destruction of coramine is about 1/10 and of cardiazol 1/16 of the amount present per minute.

6. Continuous intravenous infusion of picrotoxin simultaneously with continuous infusion of evipan permits the balancing of evipan and picrotoxin, and it is possible to work up to doses of both drugs, either of which alone would be fatal. There is a linear relation between the doses of evipan and the log. of the doses of picrotoxin which balance each other.

7. Continuous infusion of coramine superimposed on the continuous

infusion of evipan at first shows an increase in the respiratory activity, but after some time there is a gradual fall and the final depression may be greater than that produced by evipan alone. This suggests the formation of an intermediary product during coramine metabolism, which has a depressant action and is more stable than coramine.

I gratefully acknowledge my indebtedness to Professor A. J. Clark for his advice and help throughout the course of my work.

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THE CLEARANCE OF SODIUM EVIPAN. By S. C. DAS and J. RAVENTÓS. From the Department of Pharmacology, University of Edinburgh.

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THE CLEARANCE OF SODIUM EVIPAN.

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(*Received for publication 6th July* 1939.)

INTRODUCTION.

THE continuous intravenous administration of sodium evipan is now being used for the production of anæsthesia, and the experiments described below were made in order to determine the probable safety of this method. Widmark [1920] studied the clearance of narcotic drugs, whilst Widmark and Tandberg [1924] analysed the laws governing their cumulation and provided a mathematical treatment of the subject.

He also showed that two forms of drug clearance occurred:

(1) Exponential clearance with which a fixed proportion of the drug was removed per unit of time.

(2) Constant clearance with which a fixed quantity of drug was removed per unit of time.

Most drugs are cleared by the former method, and, indeed, ethyl and methyl alcohol are the only two important drugs known to be cleared by the latter method.

Widmark and Tandberg [1924] demonstrated that these two forms of clearance were of great practical importance as regards the cumulation produced by continuous or frequent administration, because in the first case cumulation rose to a maximum after which no further increase occurred, whereas in the second case there was no limit and cumulation could continue until death was produced.

The simplest method of expressing these two effects is as follows:—

In the first case if x units are given per minute and $1/y$ of the drug present is removed per minute, then cumulation will occur until the removal equals the intake, and this happens when the amount in the body is xy. This form of cumulation has been shown to occur with acetone, chloroform, and ether. In the second case if a units are given per minute and b units are removed per minute, then $a - b$ units cumulate per minute and cumulation can continue until a lethal dose is present. Methyl alcohol [Widmark and Bildsten, 1924] and ethyl alcohol [Olow, 1924] have been shown to cumulate in this manner.

It is evident that continuous intravenous injection of drugs cleared by the second method can never be safe, whereas it should be possible to administer with safety drugs that are cleared by the first method.

Various authors have used the method of continuous intravenous infusion to determine the form of clearance of non-volatile anæsthetics. Lendle [1932] showed that avertin when thus given was cleared in an exponential manner. Weese [1936] found that long-continued intravenous infusion of sodium evipan (0·5 mg./kg./min.) in rabbits produced a steady state of depression, and concluded that clearance was exponential in character. Voigt [1938] found in rabbits that a steady narcosis was produced by an initial dose of 14 mg./kg. sodium evipan followed by 0·8 mg./kg./min., and that partial recovery occurred when 0·75 mg./kg./min. was injected.

The clearance of drugs is partly dependent on their distribution in the body, and the evidence available indicates that barbiturates are distributed in a manner similar to that found by Widmark [1920] for acetone, namely, a uniform distribution throughout about 75 per cent. of the body volume.

Sodium barbitone [Koppanyi, Murphy, and Krop, 1933; Brundage and Gruber, 1937] when injected intravenously in dogs is rapidly distributed throughout nearly the whole body; about three-quarters of the dose injected leaves the blood in 1 or 2 minutes, and equilibrium is established between the blood and the tissues in 30 to 60 min. Thereafter the drug is slowly removed by excretion.

The fate of a labile substance such as sodium evipan is more difficult to establish, but Weese's [1937] analyses (intravenous injection into rabbits) show that the blood concentration falls to a level corresponding to a uniform distribution throughout the body in about 30 min. Hence the immediate fate of sodium evipan appears to be similar to that of sodium barbitone, namely, a rapid distribution throughout nearly the whole body.

These measurements do not provide much information regarding the rate of destruction of sodium evipan, but they show that during the first half-hour after administration the blood concentration of sodium evipan is influenced by two factors, namely, distribution between blood and body tissues and destruction in the body. The relative importance of these factors can only be determined with certainty by chemical analysis.

The object of the experiments in this paper was to determine whether the intensity and duration of the action of sodium evipan agreed with the hypothesis that the drug was cleared in an exponential manner. The term clearance of sodium evipan is used to imply reduction in the amount of active drug, and we have not attempted to determine whether the drug is broken down or is merely deviated by fixation by tissues other than the brain.

Clearance of Sodium Evipan by the Rabbit.

This was estimated by measuring the intensity of depression of respiration produced by continuous intravenous injection of evipan, and also by the duration of depression produced by single doses.

Methods.—The respiratory activity of rabbits was measured by Cushny's method [1913]. The animal was covered with a plethysmograph which was connected to a tambour, and the respiratory activity was estimated by multiplying the frequency and amplitude of the respiratory excursions measured on the paper. The results were expressed as the percentage of the normal respiratory activity. The animals were only partly narcotised, since the corneal reflex only disappeared when the respiratory activity was reduced to less than half the normal. Hence it was important to avoid any disturbance of the animal by noise or movement. The maintenance of a constant body temperature was very important. A thermometer was kept inserted into the rectum and a constant body temperature was maintained by an electric heater under the animal. After prolonged periods of respiratory depression there appeared to be a considerable accumulation of carbon dioxide, because when the narcotic was stopped the respiration often recovered to a higher level of activity than the normal. Hence the recovery curves are not very reliable. A considerable individual variation was observed in the response to drugs, and hence the estimations of the amount of action produced by drugs were subject to a considerable error.

The intensity and duration of the effects produced by single doses of sodium evipan were determined, and also the effects of continuous intravenous infusion. The latter was provided by a slow-turning screw acting on the plunger of a 10-c.c. record syringe. It was found undesirable to introduce fluid at a greater rate than 1 c.c. per 10 min. In many cases we used the clinical method of giving an initial massive dose and following this with a maintenance dose given by continuous infusion.

In order to interpret the effects of continuous infusion it was necessary first to know the intensity of depression produced by single intravenous doses of sodium evipan. This relation is shown in Table I.

Table I.—Relation between Dosage of Sodium Evipan and Intensity of Depression of Rabbit's Respiration.

Dosage, mg./kg. intravenous	5	10	15	20	30	50
No. of observations	3	10	18	9	5	6
Per cent. depression of respiration	15	37	49	50	62	70–100

Effect on Rabbit's Respiration of Initial Dose Followed by Continuous Injection.

This method of estimating the rate of detoxication of narcotics was introduced by Beck and Lendle [1932], who studied avertin.

The effects of 24 experiments of this type are summarised in Table II. These results are of a semi-quantitative nature because there was a wide individual variation in sensitivity. For example, the same treatment in two different animals produced 20 and 45 per cent. depressions respectively in the respiratory activity. It was, however, easy to determine whether in any animal the continuous infusion caused increase or decrease in the respiratory depression produced by the initial dose.

Table II.—Continuous Intravenous Injections of Sodium Evipan in Rabbits.

Dosage.		No. of expts.	Average depression of respiration (per cent.) produced by initial dose.	Duration of continuous infusion (hours).	Effect produced on respiration.
Rate of injection, mg./kg./min.	Initial dose, mg./kg.				
0·35	20	1	50	2·75	Slow recovery, nearly complete at 2·75 hours.
0·5	20	5	57	1·75	Initial period of recovery for 1 hour, then steady state; depression 34–45 per cent.
..	0	4	0	1·5	Fall during 1 hour to steady depression (average 30 per cent.).
0·8–1·0	20	4	66	1·25	Depression nearly constant. After 1·25 hours 55–65 per cent. depression.
	50 (slow injection)	4	70	1·5	Partial recovery to steady state 30–40 per cent. depression.
1·5	15	5	31	1–3·5	Steady increase in depression. Depression after 1 hour 45–57 per cent. (average 50 per cent.). In 3 experiments continued until respiratory arrest threatened which occurred at 1·75, 2·75, and 3·5 hours respectively.
2·0	10	1	30	1	Rapid depression 90 per cent. at 1 hour.

Table II. shows that an initial dose of 20 mg./kg. sodium evipan followed by slow intravenous infusion at the rate of 1 mg./kg./min. produced a nearly constant depression of respiration. This effect can be accounted for equally well by either of the following assumptions:—

(*a*) The rate of clearance is 5 per cent. (1/20) per min. of the drug present in the body.

(*b*) The rate of clearance is constant at 1 mg./kg./min., and does not alter when the amount in the body changes.

If the second supposition were true then it would be impossible to produce a steady depression of respiration by any rate of infusion of sodium evipan other than 1 mg./kg./min.

If 20 mg./kg. were followed by 0·5 mg./kg./min. and 1 mg./kg./min. were removed, then the whole of the initial dose would be removed in 40 min. and the animal would recover. If 15 mg./kg. were followed by 1·5 mg./kg./min. and 1 mg./kg./min. were removed, then in 60 min. about 45 mg./kg. would have accumulated in the body, a dose which is sufficient to arrest respiration (*cf.* Table I.).

Table II. shows that infusion of 0·5 mg./kg./min. results in a steady state of moderate respiratory depression, whilst infusion of 1·5 mg./kg./min. takes between 1 and 3 hours to produce threatened respiratory arrest.

The experimental results therefore indicate that a constant proportion of the drug present in the body is cleared per unit of time, and indicate a clearance of about 1/25 of the drug present per min. The rate of cumulation was calculated on this assumption, by use of the method described by Clark and Raventós [1938]. Table III. shows the

Table III.—Continuous Intravenous Injection in Rabbits.

a.		*b.*	*c.*	
Dosage.		Dose mg./kg. calculated to be present after 60 minutes if 1/25 cleared per minute.	Respiratory depression at 60 minutes.	
Initial dose, mg./kg.	Rate of injection, mg./kg./min.		Observed (Table II.).	Calculated from Column *b* and Table I.
20	0·35	9·5	30	33
20	0·5	13	40	41
20	1·0	25	61	58
15	1·5	36	50	66
10	2·0	46	90	arrest
50	1·0	28	30	60
0	1·0	23	30	55

calculated and observed figures; it will be seen that the calculations provide results which agree in most cases with the observed results.

The following experiments were made to provide as conclusive a proof as possible of the nature of the clearance of sodium evipan. An experiment of the type shown in Table II. was first made on a rabbit. A large dose (50 mg./kg.) of sodium evipan was injected cautiously so that respiratory depression just short of arrest was produced; this was followed by continuous infusion of 1 mg./kg./min. With this treatment the animal recovered to a steady state of 30 per cent. depression.

A second experiment was made on the same rabbit a few days later. In this case no initial dose was given, but only a continuous injection of 1 mg./kg./min. This caused a slow fall of respiratory activity to the same steady state that was finally reached in the previous experiment. These effects are shown in fig. 1. Three pairs of such experiments were made, with similar results.

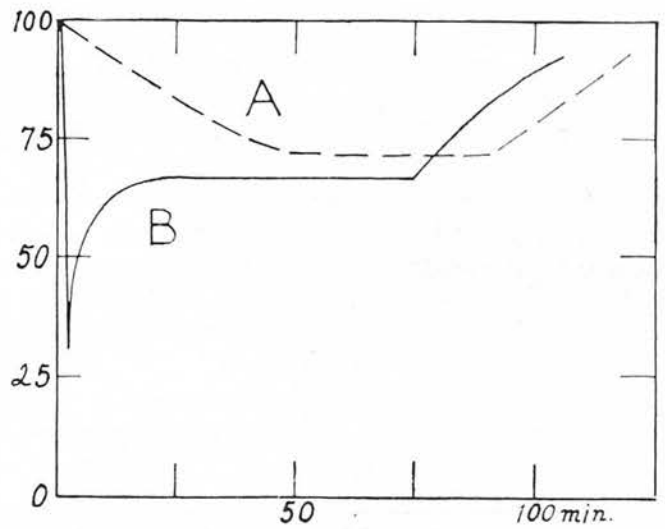

FIG. 1.—Action on rabbit's respiration of continuous intravenous infusion of sodium evipan.

Ordinate: respiratory activity as per cent. of normal; abscissa: time. A, continuous i.v.i. 1 mg./kg./min. for 90 min. B, initial dose 50 mg./kg. given over 2 min., followed by continuous i.v.i. 1 mg./kg./min. for 75 min.

The rate of continuous injection was the same in both the experiments shown in fig. 1, and the approximation to the same final steady state cannot be explained by the removal of a constant quantity of drug per minute, whereas this result is to be expected if a constant proportion of the drug present is removed per minute. These experiments therefore provide fairly conclusive proof that the proportion and not the amount of drug removed per minute is constant.

It was found possible to increase the rate of continuous infusion of sodium evipan by giving at the same time a continuous infusion of

picrotoxin. The antagonism between these drugs has been frequently described. When single doses of the two drugs were given it was found that the doses that balanced each other showed a linear relation between the dose of sodium evipan and the log. dose of picrotoxin. The relation found was that an increase of 50 mg./kg. in the dose of sodium evipan was antagonised by a fourfold increase in the dose of picrotoxin. By means of simultaneous infusion of solutions of sodium evipan and of picrotoxin it was found possible to perfuse relatively large quantities of sodium evipan. For example, in one experiment the following drugs were given. An initial dose of 10 mg./kg. sodium evipan was followed by continuous infusion of 2 mg./kg./min. for 130 min. At 45 min. 0·7 mg./kg. picrotoxin was given, and this was followed by intravenous infusion for 85 min. of 0·015 mg./kg./min.

At the end of the experiment there was 65 per cent. depression of respiration. The animal received in 130 min. a total of 270 mg./kg. sodium evipan, and, if the destruction had been steady, at 1 mg./kg./min. there would have been a cumulation of 140 mg./kg. This is nearly three times the lethal dose, and it is improbable that it could be antagonised by the amount of picrotoxin given. On the other hand, if the destruction is at the rate of 1/25 per min. the final cumulation would only be 50 mg./kg., and there is no reason why this quantity should not be antagonised by picrotoxin. This striking antagonism of sodium evipan by picrotoxin therefore provides confirmatory evidence that a constant proportion of sodium evipan is cleared per min. Our results indicate a destruction of 1/20 to 1/25 of the drug present per min., whilst Voigt's [1938] results with rabbits, analysed in the same way, indicate a destruction of 1/16 to 1/18. Kohn-Richards and Grimes (who have kindly communicated to us their results prior to publication) have studied the clearance of sodium pentothal given intravenously to rabbits, and conclude that it is cleared in an exponential manner and that 1/25 of the drug present is cleared per min. Our results are therefore in satisfactory agreement with those obtained by other workers.

Duration of Action of Single Doses.

If the depression of the rabbit's respiration by sodium evipan is dependent on the rate of clearance and if this proceeds in an exponential manner, then there should be a linear relation between duration and log. dose, and the slope of the curve should indicate the fraction of drug removed per minute.

Table IV. shows the relation between dosage and duration of depression. These results approximate to a linear relation between both dose and time and log. dose and time. Hence they do not prove the character of the clearance, but if the relation between log. dose and time be accepted then this indicates a removal per min. of 1/22 of the

drug present. The rate of clearance that accounts for the dosage-duration relation found with single doses is therefore the same as that calculated from continuous intravenous injection.

TABLE IV.—DURATION OF DEPRESSION OF RABBIT'S RESPIRATION BY INTRAVENOUS SODIUM EVIPAN.

Dose mg./kg.	10	15	20	30
No. of observations. . .	7	13	10	4
Median duration of depression of respiration (minutes) . .	18	25	33	41

CLEARANCE OF SODIUM EVIPAN BY MICE.

It was found possible to maintain narcosis in mice by continuous injection into a tail vein; a tuberculin syringe was used, and the rate of flow of fluid was 0·02 c.c. per min. The mice were kept warmed by means of an electric light.

Raventós [1938] measured the effects of intraperitoneal sodium evipan at 30° C. on large numbers of mice, and found the median hypnotic dose to be 40 mg./kg. and the median lethal dose to be 255 mg./kg. It was found (Table V.) that with intravenous injection the median hypnotic dose was about 45 mg./kg., whilst the median lethal dose was between 150 and 180 mg./kg. The latter figure depends on the rate of injection, since death can be produced by as little as 70 mg./kg. when this is injected rapidly into a vein. The figures in Table V. were obtained when the injection was given cautiously over a period of about a minute.

TABLE V.—ACTION OF SODIUM EVIPAN GIVEN INTRAVENOUSLY TO MICE. SOLUTION INJECTED OVER A PERIOD OF 1 MINUTE.

Dose, mg./kg.	No. injected.	Incidence immediate death (in 2 min.).	Incidence delayed death (2–60 min.).	Narcosis.		No narcosis.
				Incidence.	Duration in min.	
250	4	4	..	..	..	..
200	6	2	2	2	> 180	..
180	6	2	2	2	> 120	..
150	5	..	1	3	90–120	..
100	5	..	..	5	20–30	..
70	10	..	..	8	10–15	2
60	4	..	..	2	5	2
40	4	..	..	..	..	4

TABLE VI.—EFFECT OF SODIUM EVIPAN GIVEN TO MICE BY CONTINUOUS INTRAVENOUS INJECTION AFTER INITIAL INTRAVENOUS DOSE OF 70 MG./KG. CONCENTRATIONS ADJUSTED TO PRODUCE FLUID INFLOW OF 0·02 C.C./MIN.

Rate of injection, mg./kg./min.	No. of mice.	Deaths.	Effect.
20	3	3	Average time of death 20 minutes.
10	5	5	Average time of death 36 minutes.
5	3	0	Increased depth of narcosis.
3·5	3	0	,, ,, ,,
2·5	3	0	Two cases increased depth narcosis. One case narcosis unchanged.
2·0	5	0	Three cases narcosis unchanged. Two cases decreased depth narcosis.
1·5	3	0	Decreased depth narcosis.
1·0	2	0	,, ,, ,,

Table VI. shows the effects of continuous intravenous injection. A rate of 2 mg./kg./min. maintained unaltered the anæsthesia produced by an initial dose of 70 mg./kg., and this indicates a loss of $2/70 = 1/35$ per min. of the drug present. If this fraction be accepted then injection of 5 mg./kg./min. should produce a maximum cumulation of $35 \times 5 = 175$ mg./kg., and injection at this rate actually produced deep anæsthesia but not death.

Injection of 10 mg./kg./min. should raise the initial dose of 70 mg./kg. to 246 mg./kg. in 35 min., and this rate of injection caused death in this time. Injection of 1·5 mg./kg./min. should result in a gradual lowering of the drug present from 70 mg./kg. to 53 mg./kg., and a decrease in the depth of narcosis was observed with this rate of infusion.

Large numbers of animals would be needed to establish accurately the rate of clearance, but the results show that the rate of clearance in mice is less than that found with rabbits (1/25 per min.) and that it is certainly more than 1/50 per min. The relation between dosage and duration of action ought also to indicate the rate of clearance of the drug. Fig. 2 shows averages obtained from the intraperitoneal injection of more than 300 mice. The durations observed with the intravenous injection of a few mice (Table V.) also are shown for comparison.

The outstanding features of the duration of evipan narcosis in mice are the wide individual variation and the tendency for median durations to fluctuate from day to day. Hence it is very difficult to obtain reliable data. Fig. 2 suggests that the duration of narcosis is definitely shorter after intravenous than after intraperitoneal injection, and therefore the latter measurement must be affected both by the rate of absorption and the rate of clearance. The general slope of the dosage-duration

relation after intraperitoneal injection indicates, however, a loss of about 1/100 per min., and this rate is only 1/3 of that calculated from the results of continuous intravenous infusion. Neither of these methods of estimation is conclusive, but the latter appears to be the more reliable. Hence it is necessary to conclude that the relation between dosage and the duration of narcosis does not give a reliable measure of the rate of clearance of the drug.

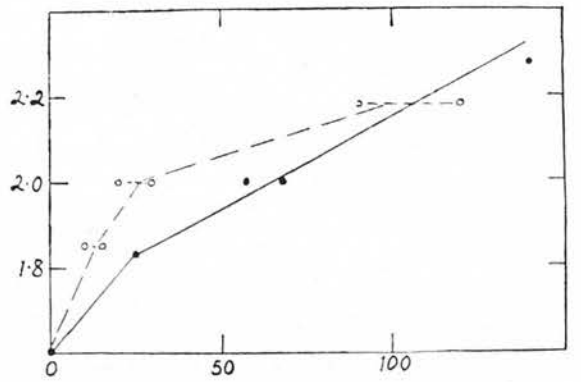

Fig. 2.—Relation between dosage of sodium evipan and duration of sleep of mice.

Ordinate: log. dosage in mg./kg.; abscissa: duration in min. Solid line: intraperitoneal injection: median results from about 300 mice. Dotted line: intravenous injection: results from a few mice.

Clearance of Sodium Evipan by Monkeys.

Storm [1935–36] measured the durations of anæsthesia produced by single doses (20 to 140 mg./kg.) of intravenous sodium evipan in 52 monkeys (Macacus). The durations ranged from 15 min. to 10 hours. The results approximate to a linear relation between dosage and duration. Over a considerable range of dosage they also approximate to a linear relation between log. dose and duration, and the slope indicates a clearance of about 1/140 per min. This result suggests that the clearance in monkeys is very much slower than in rabbits.

We measured the effect on three monkeys (*Macacus rhesus*) of an initial dose of sodium evipan followed by slow intravenous injection. A dose of 20 mg./kg. sodium evipan was found to produce a short hypnosis (20 min.). We found in one animal that when 25 mg./kg. was followed by 0·75 mg./kg./min. signs of partial recovery occurred, and that when the same initial dose was followed by 1·5 mg./kg./min. dangerous respiratory depression occurred after half an hour. These results indicate a destruction of between 1/17 and 1/33 per min. of the drug present. Other results indicated a destruction of less rather than more than 1/30 per min. The rate of clearance of evipan in the monkey appears, therefore, to be slightly less than that which occurs in the rabbit. The monkeys present the same difficulty as mice in that slow intravenous injection indicates a rate of destruction very different from that indicated by the dosage-duration relation.

Discussion.

The effects of continuous intravenous injection of sodium evipan on the rabbit provide fairly conclusive evidence that the drug is cleared in an exponential manner (*i.e.* clearance per minute of a constant proportion of the drug present). The dosage-duration relation found for the depression of the rabbit's respiration indicates the same rate of clearance as does the continuous intravenous injection (*i.e.* about 1/25 per min.). Continuous intravenous injection indicates rates of clearance in mice of 1/35 per min., and in monkeys of 1/30 per min.

The relations between dosage and duration of narcosis in mice and in monkeys indicate, however, rates of clearance respectively of 1/110 and 1/140 per min. This gross discrepancy in the results shown by the two methods may be due to a variety of causes, *e.g.* occurrence of relatively stable breakdown products of lower activity than evipan, delay in clearance of the drug from the C.N.S., etc. The problem can only be solved by chemical analysis, but our results show that the dosage-duration relation provides evidence of doubtful validity regarding the rate of clearance of evipan. This is unfortunate, because this method of estimation is much simpler than any alternative method.

Summary.

1. The effects produced on the respiratory activity of the rabbit by continuous intravenous injection of sodium evipan can be explained on the assumption that about 1/25 of the amount present in the body is inactivated every minute. These results cannot be explained on the assumption that a constant quantity of sodium evipan is inactivated per minute.

2. The relations between dosage and duration of respiratory depression in the rabbit indicate a clearance of about 1/22 per min. of the drug.

3. The effects of continuous intravenous injection in mice and monkeys indicate respective rates of clearance of 1/35 and 1/30 per min.

4. The dosage-duration of narcosis relations in mice and in monkeys indicates the much slower rates of clearance of 1/110 and 1/140 per min. respectively. The reason for the discrepancy in the results shown by the two methods is not known.

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