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ACTION OF 5,5-DIETHYL-1,3-OXAZINE-2,4-DIONE (DIOXONE) ON RESPIRATION AND CIRCULATION

BY

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The action of 5,5-diethyl-1,3-oxazine-2,4-dione (dioxone) has been studied in rats, rabbits, cats and dogs. Dioxone produced a marked stimulation of respiration in anaesthetized, decerebrate and spinal animals when given in doses that did not induce convulsions. This effect was generally accompanied by a rise in blood pressure, which was more marked in cats than in dogs and rabbits. Dioxone antagonized the respiratory and circulatory depression due to pentobarbitone. The respiratory stimulating effect of dioxone appears to be 2 or 3 times greater than that of leptazol, and comparable to that of megimide. Like leptazol, nikethamide and megimide, dioxone has no direct effect on cardiac function. Dioxone did not elicit respiratory and blood pressure changes when allowed to come in contact with the carotid sinus receptors. Dioxone enhanced the reflex excitability of bulbar centres, as demonstrated by the increase in respiratory response either to temporary common carotid artery occlusion in dogs or to electrical stimulation of the central cut end of Hering's nerve in the cat. Dioxone also reduced the inhibition of respiration induced by electrical stimulation of the central cut end of the vagus nerve. Whether this central action of dioxone is direct or not cannot at present be elucidated, though a section at intercollicular level did not prevent the respiratory stimulation produced by this substance.

Dioxone (5,5-diethyl-1,3-oxazine-2,4-dione) is a new compound, synthesized by Testa, Fontanella, Cristiani & Gallo (1959), that possesses convulsant properties (Maffii & Silvestrini, 1961) and antagonizes the toxic and lethal effect of barbiturates and other central nervous system depressants.

During the investigation of the convulsant activity of dioxone (Maffii, Dezulian & Silvestrini, 1961), as well as in the study of its protective effect against acute barbiturate intoxication (Maffii & Serralunga, 1961), it was observed that this substance markedly stimulated the rate of respiration. It appeared therefore of interest to determine the effect of dioxone on respiration as well as on the circulation. In the present paper we report the experimental results of such a study that has been carried out on different animal species in comparison with other analeptics.

METHODS

The experiments were done on Wistar rats (180 to 210 g), guinea-pigs, rabbits, cats and dogs. To study the changes in rate of respiration, rats were given pentobarbitone sodium

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by intraperitoneal injection in doses of 50 mg/kg. A group of animals was kept as controls, while other groups were given various subcutaneous doses of dioxone or leptazol 5 min after the administration of the barbiturate. In other experiments, phenobarbitone was used in doses of 120 mg/kg intraperitoneally, and the analeptics were administered 30 min later. The respiratory frequency was measured every 10 min for 2 hr, by direct observation of the animals.

Oxygen consumption. For the oxygen consumption studies, male rats fasted for 15 hr were given pentobarbitone sodium intraperitoneally in a dose of 50 mg/kg, and then put individually into the thermoregulated chamber of a closed-circuit apparatus. The system was filled with air, carbon dioxide being absorbed by passing the air through two bottles containing 30% potassium hydroxide solution and a filter made of quicklime. The oxygen consumption was evaluated through the vol. reduction of the reservoir. The reduction in vol./given time was expressed as dry gas, at normal conditions (0° C and 760 mm Hg), and then reduced to ml. of oxygen/100 g of body weight/min. Each animal received in different stages at 3 days' interval the following treatments: (*a*) pentobarbitone intraperitoneally alone, (*b*) pentobarbitone and 5 min later the analeptic by subcutaneous injection. The cross-over experiment was designed on a Latin square basis. Measurements were always started 10 min after the animal was injected with pentobarbitone so that the oxygen consumption was determined during the period from the 10th to the 30th min after the barbiturate. Animals which awakened or had convulsive attacks during this period because of the effect of the stimulant agents were discarded.

Respiration. The respiratory movements in rabbits, cats and dogs were recorded by 3 different devices: (*a*) Marey tambours connected with the side arm of a tracheal cannula; (*b*) the respirometer of Anderson (1953) connected with a tracheal cannula in order to evaluate tidal air (both with kymograph recordings); (*c*) a pneumometer fixed to the thorax of the animal, and connected with a pressure transducer, the signal of which was amplified by a DC amplifier and recorded on a multichannel electronic (Sanborn) recorder.

Cardiovascular system. Blood pressure of rabbits, cats and dogs was recorded on a kymograph through mercury manometers, as well as on an electronic recorder, through a DC preamplifier, by means of a pressure transducer connected with cannulae inserted either into the femoral or into the carotid artery. Strength of contraction of the dog's heart was recorded by means of a strain gauge arch (Boniface, Brodie & Walton, 1953) fixed to the myocardium of the left ventricle, according to the method described by Cotten & Bay (1956). Operations were performed in animals under pentobarbitone anaesthesia and under artificial respiration provided by a Palmer pump.

Decerebrate and spinal preparations. Decerebration was done under ether anaesthesia by sectioning the mesencephalon at the intercollicular level on the dorsal side and at the level of mammillary bodies on the ventral side. Both common carotid arteries were occluded at the moment of the section. Experiments were started at least 1 hr after the section. In spinal preparations the section at C2 was preceded by the injection of a few drops of 1% procaine solution into the medulla in order to reduce traumatic shock. The excitability of bulbar centres was studied in dogs through the responses elicited by common carotid occlusion and stimulation of the cut central end of the vagus nerve and through the carotid sinus nerve (Hering's nerve) stimulation in cats. Two doses of dioxone were usually administered: one that did not produce appreciable respiratory stimulation, and the other that increased the rate and/or depth of respiration. In dogs under pentobarbitone anaesthesia carotid sinus reflexes were elicited by occlusion of one common carotid for 30 sec. This treatment produces an increase in rate and depth of respiration, a rise of arterial pressure and an increase in heart rate (Heymans & Neil, 1958).

Temporary inhibition of respiration was produced in dogs under pentobarbitone anaesthesia by stimulation of the central end of the vagus nerve sectioned at the neck, with square-wave currents (frequency, 50/sec for 20 sec). Intensities of current were used that were slightly greater than threshold.

Stimulation of the carotid sinus nerve. In cats under pentobarbitone anaesthesia (60 mg/kg intraperitoneally) the carotid sinus nerve was dissected clear of surrounding structures and cut. The central end was stimulated with square-wave stimuli delivered through an electronic stimulator. The duration of stimulation was 30 sec; each single square stimulus had a duration of 0.26 msec. The frequency was varied from 2 to 20 stimuli/sec in order to produce different effects on respiration.

Carotid sinus chemoreceptors. To study the direct effect of dioxone on the carotid sinus chemoreceptors of cats, the lingual and thyroid arteries were cannulated and an injection made alternately in one and then the other artery. Lobeline in doses of 100 and 200 μg was injected to check the response of the preparation.

Isolated guinea-pig auricles were prepared according to the method of Giotti & Nardini (1953).

Solutions. Dioxone was dissolved in distilled water at concentrations from 0.5 to 1%.

RESULTS

Effects on respiratory function

Rats. The effect of dioxone on the frequency of respiration depressed by intraperitoneal pentobarbitone and phenobarbitone was studied by measuring the number of respiratory movements/min every 10 min. Dioxone and leptazol were administered 5 min after the barbiturates.

Fig. 1 shows the stimulation of respiration produced by dioxone in animals given pentobarbitone 50 mg/kg intraperitoneally. Within 60 to 85 min dioxone

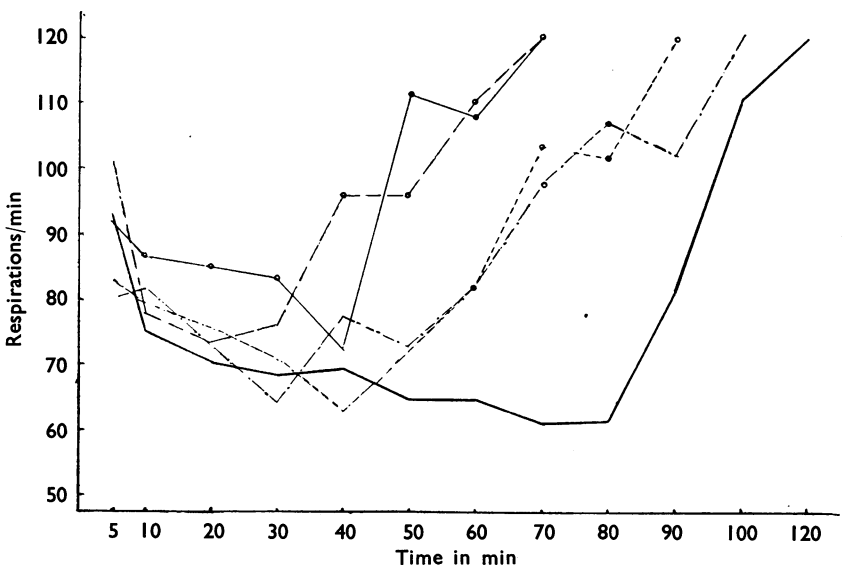

Fig. 1. Effects of dioxone and leptazol on the respiratory rate in rats under pentobarbitone sodium anaesthesia. The trial was performed on 5 groups of 5 rats which were injected intraperitoneally with pentobarbitone sodium (50 mg/kg); 5 min later the 5 groups were injected subcutaneously with 0.9% sodium chloride solution (———), dioxone 25 mg/kg (○ - - - ○), dioxone 50 mg/kg (○— — —○), leptazol 50 mg/kg (○— · — · —○) and leptazol 100 mg/kg (○——○) respectively. The respiratory rate was observed until the animals awakened. Ordinate: respirations/min. Abscissa: time in min. The circlets shown in the figure indicate that the result was significantly different from the control (P=0.05) as calculated by the t test.

antagonized almost completely the depressant effect of pentobarbitone in subcutaneous doses of 25 and 50 mg/kg. Leptazol was much less effective, as the doses required were twice as large as those of dioxone. Furthermore, leptazol was found to be completely ineffective at the lower dose studied (25 mg/kg). Dioxone was definitely effective at a dosage of 25 mg/kg, although its action was brief. At a dose of 50 mg/kg the activity of dioxone was greater and was exerted for at least 1 hr. Leptazol was completely inactive at a dose of 25 mg/kg, and in doses of 100 mg/kg it appeared less effective both in intensity and duration than dioxone at 50 mg/kg. It is obvious that in the case of phenobarbitone the depression lasts longer than the effect of dioxone, and the general picture of the results is quite different from that obtained in animals given pentobarbitone, the duration of action of which is about equal to that of dioxone.

In order to ascertain whether the stimulating effect of dioxone on respiration results in an increase in oxygen consumption, experiments were carried out on rats. Animals given pentobarbitone in doses of 50 mg/kg intraperitoneally showed a reduction of 50% in the oxygen consumed/min/100 g body weight. As shown in Table 1, dioxone (25 and 50 mg/kg) restored to some extent the oxygen consumption which had been depressed by pentobarbitone. Leptazol, tested for the same activity under the same conditions, appeared obviously less active than dioxone.

TABLE 1

OXYGEN CONSUMPTION OF RATS AFTER PENTOBARBITONE AND ANALEPTIC AGENTS

Observations were made over a 20 min period. Pentobarbitone was injected intraperitoneally (i.p.) and dioxone and leptazol subcutaneously (s.c.)

Treatment	Dose mg/kg	No. of determinations	ml. oxygen/min/100 g body weight	s.e.	% changes
Controls (awake)	—	12	3·19	0·08	—
Pentobarbitone	50 i.p.	4	1·55	0·07	—
Pentobarbitone+ dioxone	50 i.p. + 25 s.c.	4	1·89	0·10	21·93
Pentobarbitone	50 i.p.	4	1·57	0·06	—
Pentobarbitone+ dioxone	50 i.p. + 50 s.c.	4	2·41	0·07	53·51
Pentobarbitone	50 i.p.	4	1·79	0·07	—
Pentobarbitone+ leptazol	50 i.p. + 100 s.c.	4	2·68	0·35	49·72

Rabbits. In animals anaesthetized with urethane (1 mg/kg intraperitoneally), dioxone in doses ranging from 3 to 5 mg/kg injected intravenously produced an increase in the respiratory amplitude in 2 out of 4 animals, and an increase in the respiratory frequency in 3 out of 4 animals, for a period of 12 to 20 min. Leptazol produced similar effects at a dose of 10 mg/kg.

A second group of animals was anaesthetized with pentobarbitone injected intravenously in doses of 20 and 30 mg/kg, which produced obvious depression of both the frequency and amplitude of respiration ; 10 and 20 mg/kg of dioxone appreciably

increased the frequency and amplitude of respiration, and similar effects were obtained with 30 mg/kg of leptazol.

In animals treated with morphine 10 mg/kg intravenously, dioxone in doses as low as 4 mg/kg stimulated respiration, but also produced marked signs of motor excitation, and even clonic convulsions in some cases. Electroencephalographic registrations, performed simultaneously, demonstrated that the convulsant effects of

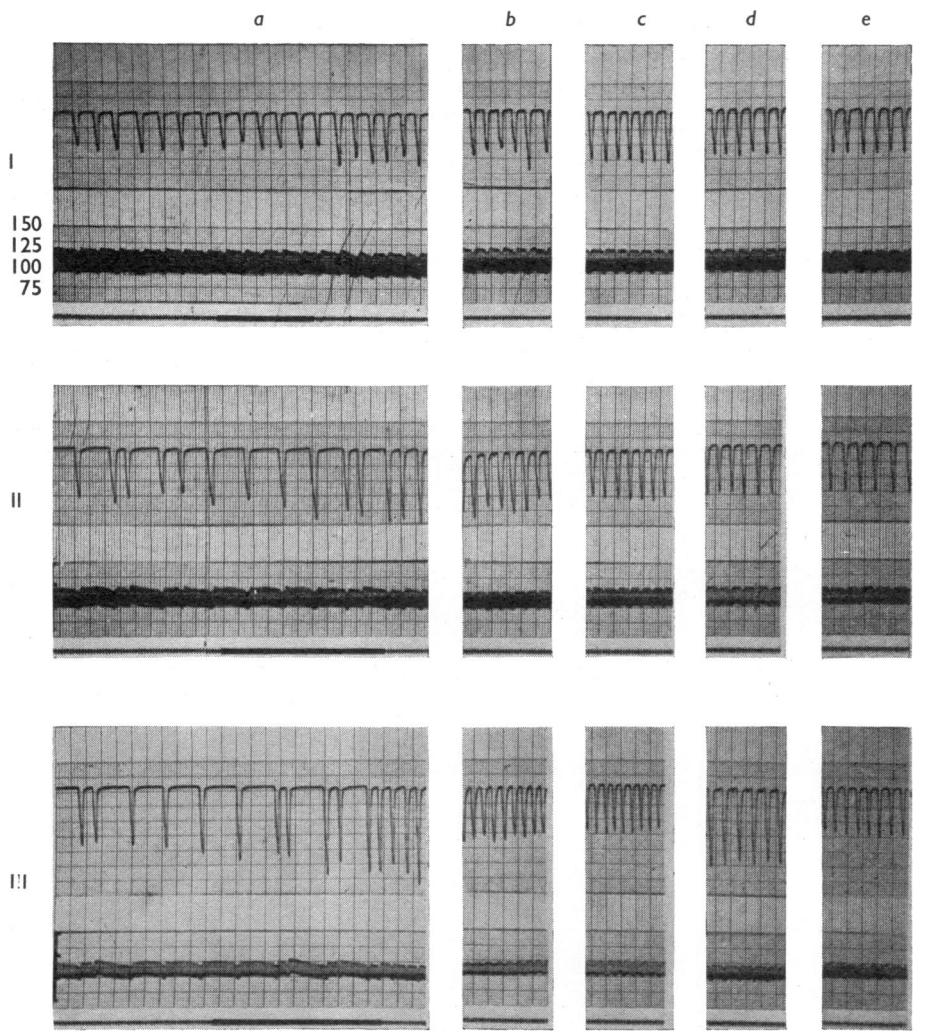

Fig. 2. Dog anaesthetized with pentobarbitone sodium (40 mg/kg intravenously). Records from above downwards: pneumogram recorded by pressure transducer through thoracic pneumograph (inspiration downwards); carotid blood pressure recorded by a pressure transducer and signal/time marker (1 sec). The effects of dioxone injected intravenously: I, 2 mg/kg 15 min after pentobarbitone; II, 4 mg/kg 15 min after a further 15 mg/kg of pentobarbitone; III, 8 mg/kg 15 min after a further 10 mg/kg of pentobarbitone. The intervals between the tracings horizontally were 5 min. There was a 40 min interval between I and II and between II and III.

dioxone were greatly increased in animals pretreated with morphine ; this has also been observed following leptazol 10 mg/kg. Existing data show that there is synergism between morphine and leptazol in the production of convulsions (Hazleton & Koppanyi, 1941).

Cats. The experiments were performed on animals anaesthetized with chloralose (70 mg/kg intraperitoneally), urethane (1 g/kg intraperitoneally) and pentobarbitone (60 mg/kg intraperitoneally). In chloralosed animals 2.5 mg/kg of dioxone produced a clear respiratory stimulation, though diffuse muscular clonus was also observed. In animals treated with urethane, higher doses, namely, 5 to 10 mg/kg, were necessary and produced essentially an increase in amplitude. In this case the convulsive phenomena occurred only at much higher doses. In cats given pentobarbitone the effects of dioxone were found to be dependent on the depth of anaesthesia and the depression of the respiratory function ; stimulation was obtained with 4 to 8 mg/kg.

Dogs. In animals deeply anaesthetized with 60 mg/kg of pentobarbitone injected intraperitoneally, dioxone caused a moderate increase of the respiratory frequency and amplitude in intravenous doses as low as 2 mg/kg (Fig. 2). With 6.8 and 10 mg/kg or more the effect was more obvious and lasted for 15 to 30 min (Fig. 3).

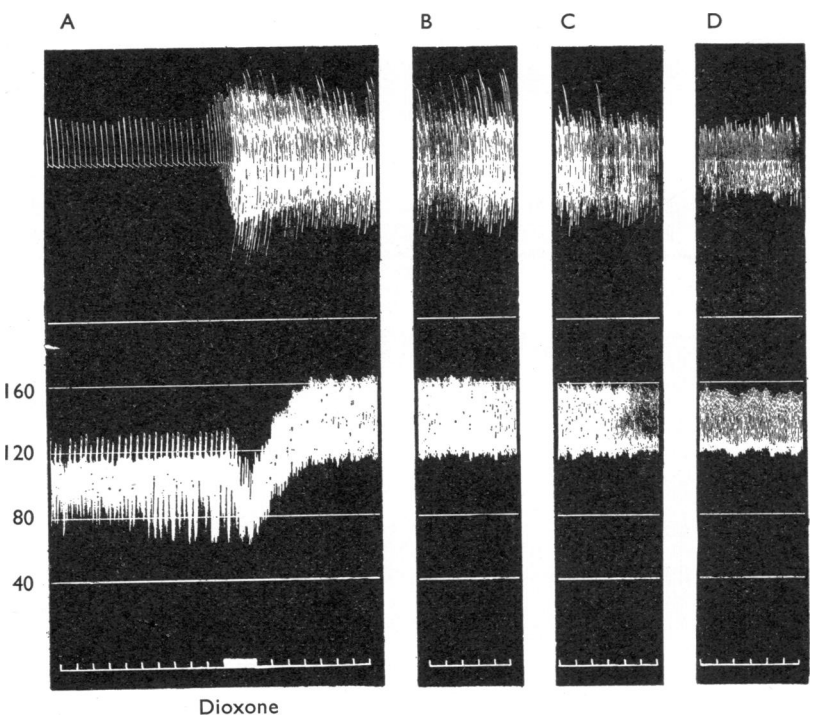

Fig. 3. Effect of a high dose of dioxone on respiration and blood pressure of dog under pentobarbitone sodium 40 mg/kg injected intravenously. A: At the signal dioxone 15 mg/kg was injected intravenously. The interval between A and B was 5 min, between B and C 10 min, and between C and D 30 min. Time marker, 1 min.

In general, the frequency and amplitude were found to be increased to the same extent. The actions of dioxone and megimide on the respiration of pentobarbitone-depressed dogs were found to be equivalent.

Effect of dioxone on decerebrate animals

Decerebrate rabbits. Dioxone in intravenous doses of 2.5 to 7.5 mg/kg produced stimulation of respiration in 7 out of 8 decerebrate rabbits. In some cases there was, a few sec after the injection, an increase in amplitude, and in other animals an increase of frequency that was usually accompanied by increase in rigidity and slight motor stimulation.

Decerebrate cats. The activity of dioxone given in doses of 2.5 to 5 mg/kg to 8 decerebrate cats appeared greater than in decerebrate rabbits, and the increase in the rate of respiration was often accompanied by increase in the amplitude of single movements, as demonstrated by the increase in tidal air. Thus the effect of dioxone in decerebrate cats appears to be similar, or even greater, than in deeply anaesthetized animals.

Leptazol has little or no stimulating effect on respiration of decerebrate animals, even when given in doses twice those of dioxone. Megimide, on the contrary, is still effective in decerebrate animals in doses similar to dioxone.

Decerebrate dogs. Dioxone 5 to 7 mg/kg caused a slight augmentation of respiration in 4 out of 6 decerebrate dogs. In 2 animals receiving 2.5 mg/kg, dioxone appeared ineffective. Contrary to what occurred in cats, mesencephalic section of the brain stem appeared to reduce the respiratory stimulation produced by dioxone.

Effects of dioxone on blood pressure

The experiments were carried out on rabbits, cats and dogs, both anaesthetized and decerebrate. In the different species the common feature of the action of dioxone may be summarized as follows. In doses from 2 to 10 mg/kg intravenously dioxone produced generally an increase in blood pressure, though this action was not as constant as the effect on respiration, and depended upon the pressure level before treatment, being small or absent in lightly anaesthetized animals, with an initial systolic pressure of 110 to 140 mm of mercury. In deeply anaesthetized animals with an initial blood pressure of less than 100 mm of mercury, dioxone (3 to 10 mg/kg) produced a 15 to 40% increase in the pressure level. With higher doses, when muscular twitches or clonic convulsions were evident, there was always an increase in blood pressure. The rise in blood pressure was generally prolonged, though when hypotension was marked, due to high doses of a barbiturate, the effect of dioxone (5 to 10 mg/kg intravenously) usually lasted for 3 to 6 min, and further administration was necessary in order to maintain satisfactory levels. A brief analysis of the results follows.

Rabbits. The blood pressure level was registered in unanaesthetized and curarized animals, in animals treated with morphine, as well as in spinal and decerebrate animals. Respiration and, in some cases, electroencephalographic records were registered at the same time as the haemodynamogram. In unanaesthetized rabbits,

dioxone in a dose of 5 mg/kg intravenously produced a rise in blood pressure of 40 to 80% and at the same time convulsive phenomena in the electroencephalogram at cortical level, and marked respiratory stimulation. Greater effects were usually seen after doses of 8 to 10 mg/kg. Both bemegride and leptazol exert similar effects, the former in the same dose range as dioxone, and the latter in doses of 15 to 20 mg/kg.

When repeated doses of dioxone were administered at intervals allowing a reduction of 50% of the hypertensive response, the rise in blood pressure reappeared after each injection until the fourth or fifth one, after which further administration was unable to produce changes in the blood pressure. Doses of 5 and 10 mg/kg intravenously were repeated 10 times at intervals, respectively of 10 and 15 min in 3 rabbits each, without causing death even when severe convulsions were present.

In curarized rabbits (tubocurarine 0.5 mg/kg intramuscularly) the effects of dioxone did not substantially differ from those described for unanaesthetized animals. In animals given morphine (10 mg/kg intravenously) lower doses (2 to 4 mg/kg) of dioxone elicited the hypertensive as well as the convulsive response, together with respiratory stimulation. In decerebrate rabbits dioxone produced only slight increase in blood pressure in 2 out of 5 animals, and in 2 cases the

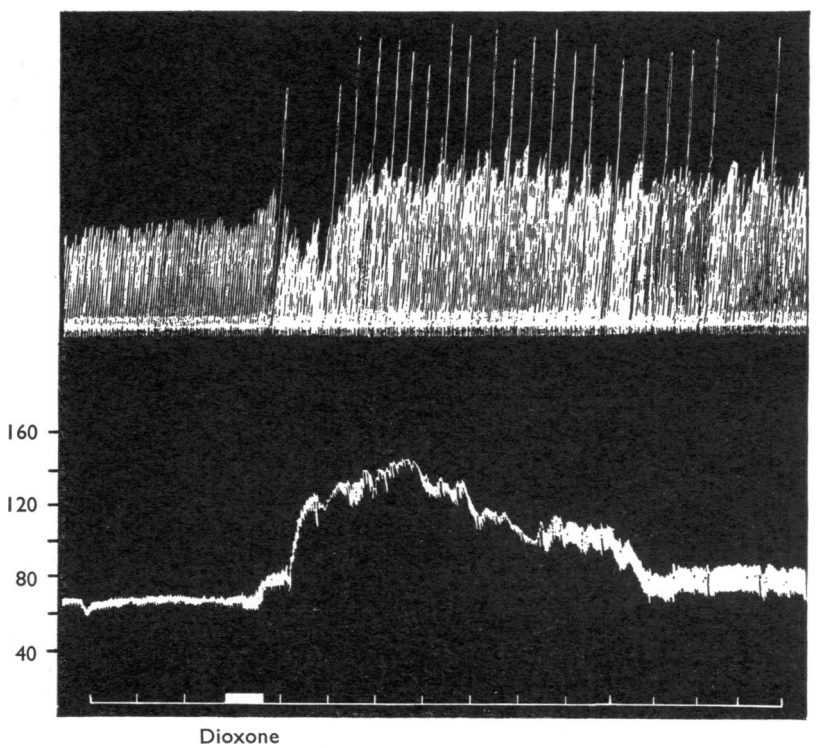

Fig. 4. Effects of intravenous dioxone (5 mg/kg) on the decerebrate cat. Records from above downward: tidal air recorded through an Anderson respirometer, carotid blood pressure recorded by mercury manometer and signal/time marker (1 min).

stimulation of respiration caused by doses of 5 and 7.5 mg/kg intravenously was accompanied by some lowering of blood pressure.

Cats. In curarized animals (tubocurarine 0.6 mg/kg intramuscularly) dioxone produced transitory hypertension in doses (5 to 10 mg/kg intravenously) which caused convulsions, as demonstrated by electroencephalographic recordings. In cats anaesthetized with pentobarbitone sodium (60 mg/kg intraperitoneally) dioxone produced a rise in blood pressure even in a dose of 2 mg/kg intravenously. This effect lasted usually for 15 to 20 min and was not always accompanied by respiratory stimulation. With higher doses the hypertensive effect was not more marked, but it was accompanied by an increase in rate and/or amplitude of respiration, and with doses higher than 8 to 12 mg/kg by convulsions. In decerebrate cats dioxone still produced a rise in blood pressure (Fig. 4) similar to that observed in anaesthetized ones. In spinal cats, dioxone (2 to 10 mg/kg) had very inconsistent effects on blood pressure, and produced in 4 out of 6 animals a lowering which disappeared either after sectioning both vagi or after atropine 0.5 mg/kg intravenously.

Dogs. In animals in deep pentobarbitone anaesthesia (40 mg/kg intravenously) dioxone produced a smaller rise in blood pressure than that seen in cats. The rise seemed to be directly related to the degree of respiratory stimulation. In decerebrate dogs dioxone had approximately the same slight effects as observed in anaesthetized animals.

Effects of dioxone on the heart

Dogs. The effects of dioxone on cardiac contractile force and electrocardiogram were studied in dogs under deep pentobarbitone anaesthesia (40 mg/kg intravenously in repeated doses) and under artificial respiration. Dioxone in doses of 1 to 3 mg/kg had no appreciable effect. Higher doses (9 to 18 mg/kg) slightly raised the blood pressure, but had no effect on the heart. Only in particularly depressed animals was the rise in blood pressure produced by dioxone secondarily followed by slightly increased contractions. In some experiments a comparative study was carried out with leptazol, megimide and nikethamide, the doses being adjusted so that leptazol was administered in the same range as dioxone, leptazol from 10 to 30 mg/kg and nikethamide from 10 to 40 mg/kg. Direct comparisons in the same animal demonstrated that the three substances had no significant action on the heart.

Isolated guinea-pig auricles. Nikethamide, megimide, leptazol and dioxone, given in concentrations of 1 to 20 μg/ml., had no inotropic or chromotropic effect on this preparation.

Effects of dioxone on the excitability of bulbar centres

Response to common carotid occlusion. It is known that occlusion of the common carotid artery for 30 sec causes a rise in blood pressure and an increase in rate and depth of respiration (Heymans & Neil, 1958). When dioxone was given in doses of 2 mg/kg intravenously, which in the dog produced little stimulation of respiration, the increase in rate and depth of respiration induced by common carotid occlusion appeared to be potentiated for some time, though there was

little or no effect on the pressure response. Following 4 mg/kg intravenously the respiratory rate was increased by dioxone itself, and the changes in the respiratory response to common carotid occlusion became difficult to evaluate.

Response to central stimulation of the vagus in the dog. The temporary inhibition of respiration produced by electrical stimulation of the central end of the vagus was slightly reduced in dogs given dioxone at 2 mg/kg intravenously. Higher doses (4 mg/kg intravenously), which produced some stimulation of respiration, usually abolished for some 10 min the inhibition of respiration caused by stimulation of the vagus.

Response to central stimulation of Hering's nerve in the cat. Electrical stimulation of the central end of Hering's nerve of the cat induces different effects according to the frequency of stimulation. As demonstrated by Douglas & Schaumann (1956), by varying the frequency from 2 to 40 stimuli/sec, it is possible to elicit a respiratory response and a pressure rise that become clear at 4 or 6 stimuli/sec and are then greater in proportion to the frequency of stimulation. In 4 cats anaesthetized with pentobarbitone, the effects of stimulation of the central end of Hering's nerve at various frequencies were studied before and after the administration of dioxone in doses that did not affect respiration. As shown in Fig. 5, under the effect of dioxone the threshold of frequency of stimulation needed to elicit a minimal response of respiration was lowered and the effect of supraliminal stimulation enhanced. When doses of dioxone were given high enough to produce an increase in rate and depth of respiration (4 mg/kg intravenously), the response to Hering's nerve stimu-

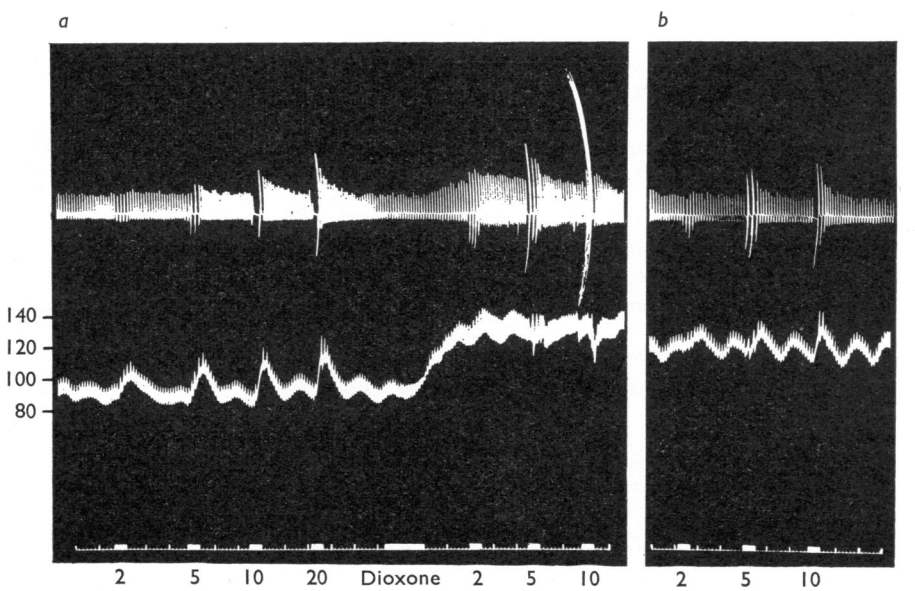

Fig. 5. Effects of dioxone on the respiratory and blood pressure response to the stimulation of Hering's nerve in the anaesthetized cat. Signal/time marker (1 min). After stimulation with frequencies of 2, 5, 10, and 20/sec, dioxone, 4 mg/kg, was injected intravenously. Then the stimulation was repeated at 2, 5, and 10/sec. Interval between A and B was 60 min.

lation appeared very marked. However, the hypertensive response was not changed after both dose levels of dioxone.

Lack of peripheral action of dioxone on carotid sinus

In order to exclude a possible action of dioxone on respiration and blood pressure by a peripheral mechanism, for example, by stimulating the receptors of glomus caroticus, experiments were performed on 4 cats by injecting the drug in the lingual and in the thyroid artery: in the first case the drugs pass through the carotid bifurcation, and in the second not. Dioxone in doses of 2.5 mg/kg did not act differently whether or not it came in contact with the carotid glomus. Lobeline in a dose of 100 and 200 μg produced in the same animals a clear stimulation of respiration only when injected in the thyroid artery.

Two dogs whose bifurcation of carotids was perfused for 15 min with Ringer containing a concentration of dioxone as high as 1% did not show any increase in respiration. Lobeline at a concentration of 50 μg/ml. produced a definite effect after a few sec.

DISCUSSION

The results show that dioxone produces a marked stimulation of respiration. This action is very clear in animals depressed by barbiturates or other anaesthetics and may be observed even with doses of 2 mg/kg intravenously. Dioxone produces changes both in frequency and amplitude of respiration and is able to restore to some extent the oxygen consumption depressed by pentobarbitone. The effect on respiration is still present in decerebrate cats and rabbits but is greatly decreased in decerebrate dogs. Together with the increase in respiration, dioxone produces a rise in blood pressure that is usually inversely proportional to the pretreatment levels.

This rise in blood pressure, however, is less evident in rabbits and dogs. In fact, in those animals greater doses must be employed to cause a rise in blood pressure than are necessary to produce augmentation of respiration. The hypertensive effect of dioxone is not modified by decerebration in cats, but is variable in decerebrate dogs and rabbits.

In spinal cats dioxone tends to lower the blood pressure, and this action is inhibited either by section of both vagi or by atropinization. This finding suggests that the stimulation produced by dioxone on bulbar centres results in generalized discharges involving the main efferent paths, and that the hypertension observed in intact animals is due to the prevalence of sympathetic over vagal stimulation. Submedullary sections, by abolishing the sympathetic component, allow the vagal stimulation to appear. This hypothesis is also in agreement with the finding of brief episodes of hypotension occasionally observed in some animals immediately after, or during, the intravenous injection of dioxone and rapidly followed by the usual rise in blood pressure.

As far as the mechanism of action is concerned, it can be stated that the effects of dioxone on respiration and blood pressure are due to its action on the central nervous system. By studying the reflex excitability of the bulbar centres it has

been demonstrated that dioxone, in doses that do not affect respiration, or stimulate it very little, can produce an increase in the respiratory reflex stimulation due to a rapid hypotension at the level of carotid glomus, and also an augmentation of the reflex response due to electric stimulation of Hering's nerve.

On the other hand, the period of apnoea which may be produced in dogs by stimulation of the central portion of the sectioned vagus nerve may be reduced or abolished for a short time following the administration of dioxone. The hypertensive response to common carotid occlusion is usually unmodified or little increased. It seems likely, on the basis of these findings, that dioxone acts on centres that regulate respiration both by increasing the excitability of these structures to reflex stimulation and by insulating them from inhibitory effects. Whether this action of dioxone is direct or mediated through the effect on other parts of the central nervous system requires further elucidation. However, the results obtained in decerebrate animals demonstrate that the connexion of the bulbar centres with structures lying at higher than mesencephalic levels is not strictly necessary for this action of dioxone. This fact, which has been clearly established in cats, is not so definite in rabbits and dogs.

In dogs, respiratory centres are more susceptible than vasomotor centres to stimulation by dioxone, lower doses being necessary to elicit increase in respiration than to produce a rise in blood pressure.

In cats the pattern of the action of dioxone differs substantially because the hypertensive effect of the drug may sometimes be demonstrated in the absence of respiratory stimulation. However, in these conditions a lowering of the threshold for reflex stimulation of the respiratory mechanism through the stimulation of Hering's nerve can be demonstrated. On the basis of studies by Kissel & Domino (1959) on the influence of blood pressure changes on the reflex excitability of the central nervous system, we can exclude the possibility that the rise in blood pressure caused by dioxone in these circumstances is responsible for the increase in the excitability of respiratory centres.

Our experiments have confirmed the lack of any direct influence of leptazol and nikethamide on cardiac function, in agreement with the findings of Goodman & Gilman (1955) and Krop (1944). Moreover, megimide and dioxone have also no significant effect on the heart. This emphasizes the limits of the practical use of analeptic drugs, though within these limits it would appear that dioxone has pharmacological properties of interest in therapeutics.

REFERENCES

ANDERSON, F. F. (1953). Quantitative volumetric respiratory recorder. *Arch. int. pharmacodyn.*, **94**, 460–461.

BONIFACE, K. J., BRODIE, O. J. & WALTON, R. P. (1953). Resistance strain gauge arches for direct measurement of heart contractile force in animals. *Proc. Soc. exp. Biol., N.Y.*, **84**, 263–266.

COTTEN, M. DE V. & BAY, E. (1956). Direct measurement of changes in cardiac contractile force. *Amer. J. Physiol.*, **187**, 122–134.

DOUGLAS, W. W. & SCHAUMANN, W. (1956). A study of the depressor and pressor components of the cat's carotid sinus and aortic nerves using electrical stimuli of different intensities and frequencies. *J. Physiol. (Lond.)*, **132**, 173–186.

GIOTTI, A. & NARDINI, F. (1953). Studi sull'attività degli atrii isolati in varie condizioni sperimentali e durante l'azione di vari gruppi di farmaci. Nota I: Tecnica Sperimentale. *Arch. ital. Sci. Farmacol.*, **3**, 275–286.

GOODMAN, C. S. & GILMAN, A. (1955). *The Pharmacological Basis of Therapeutics*, 2nd ed., p. 328. New York: Macmillan.

HAZLETON, L. W. & KOPPANYI, T. (1941). The combined action of morphine and central stimulants and its relation to the treatment of morphine poisoning. *Anaesthesiology*, **2**, 427–442.

HEYMANS, C. & NEIL, E. (1958). *Reflexogenic Areas of the Cardiovascular System*, p. 35. London: Churchill.

KISSEL, J. W. & DOMINO, E. F. (1959). Effects of controlled progressive hypotension on some spinal reflexes in the cat. *Amer. J. Physiol.*, **196**, 59–64.

KROP, S. (1944). Influence of "heart stimulants" on the contraction of isolated mammalian cardiac muscle. *J. Pharmacol. exp. Ther.*, **82**, 48–62.

MAFFII, G., DEZULIAN, V. M. & SILVESTRINI, B. (1961). A new analeptic: 5,5-diethyl-1,3-oxazine-2,4-dione (dioxone). I, Toxicity and convulsant activity. *J. Pharm. (Lond.)*, **13**, 244–253.

MAFFII, G. & SERRALUNGA, M. G. (1961). A new analeptic: 5,5-diethyl-1,3-oxazine-2,4-dione (dioxone). II, Antagonism towards some C.N.S. depressants. *J. Pharm. (Lond.)*, **13**, 309–317.

MAFFII, G. & SILVESTRINI, B. (1961). Rapporti tra struttura e attività farmacologica. *Farmaco (Ed. Scient.)*, **16**, 39–57.

TESTA, E., FONTANELLA, L., CRISTIANI, G. & GALLO, G. (1959). 5,5-Disubstituted dihydro-1,3-oxazine-2,4 diones. Research on compounds active on central nervous system, XII. *J. org. Chem.*, **24**, 1928.