A NEW ANALEPTIC: 5,5-DIETHYL-1,3-OXAZIN-2,4-DIONE (DIOXONE)

I. Toxicity and Convulsant Activity

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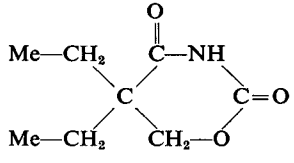
Dioxone (5,5-diethyl-1,3-oxazin-2,4-dione) is a new substance possessing convulsant properties qualitatively similar to leptazol and bemegride. Its convulsant properties have been studied quantitatively in mice and rats. By comparison with the other two analeptics, dioxone shows the highest ratio of lethal to convulsive doses. Dioxone is active orally in mice, rats and dogs. Experiments on spinal cats and rabbits have shown that it does not act on the spinal cord. In the decerebrated animals its effects appear limited and inconsistent. It has a relatively low toxicity, is well tolerated by rats and dogs when administered for up to 6 months either subcutaneously or orally.

The discovery of the convulsant properties of some compounds belonging to a series of 5,5-disubstituted 1,3-oxazin-2,4-diones has been reported by Maffii and Silvestrini (1961) and of these 5,5-diethyl-oxazin-2,4-dione, Dioxone, proved to be most active.

Contrary to the actions of 5-phenyl-5-alkyl-1,3-oxazin-2,4-diones which prevent experimentally induced seizures in mice, dialiphatic substituted derivatives including dioxone produce clonic and clonic-tonic convulsions.

The antagonism exhibited by some substances of the former group both towards dioxone and leptazol suggested a similarity between the convulsant activity of these two substances.



Dioxone

We now report the results of the pharmacological investigation of the convulsant activity and toxicity of dioxone in different species.

Materials and Methods

Dioxone is a white crystalline substance that melts at 97°, and is readily soluble in ethanol, ether, chloroform and propylene glycol. A 1 per cent aqueous solution may be obtained at room temperature giving a pH of

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4·3, and, with warming to 50°, 2 per cent aqueous solutions may be prepared.

CF-1 Mice, CF-Wistar rats, mongrel dogs, domestic cats and rabbits were used in this work.

In acute toxicity studies mice and rats within a limited weight range were chosen, and the volume of injected solution was kept constant. Intravenous administration was at a rate of 0·1 ml./sec. The volumes administered were in mice per 20 g. weight, 0·5 ml. i.v., 0·2 ml. i.p. and orally; in rats per 200 g. weight: 0·8 ml. s.c., 0·4 ml. i.p. and orally. Four or 5 doses were used and 10 animals for each dose. Deaths occurring within 72 hr. were considered to be due to the compound.

In subacute and chronic toxicity studies in rats, complete blood counts were made before, and every 20 days during the treatment. With dogs receiving dioxone in chronic toxicity tests urine analysis was made every week, glucose and nitrogen blood serum levels every two weeks, as well as Takata-Ara reaction, paper-electrophoretic analysis of plasma proteins, complete blood cell count and prothrombin time tests.

In studies on convulsant activity, the 50 per cent effective dose was determined in rats and mice by administering 3–5 doses of the compounds to groups of 10 animals each and then observing them continuously for clonic and tonic seizures. *For the studies on decerebrated animals*, section of the brain stem was made under ether anaesthesia at intercollicular level. In spinal preparations the section was preceded by injection of a few drops of 1 per cent procaine solution into the medulla to reduce traumatic shock.

The method of Litchfield and Wilcoxon (1949) was followed for estimating the LD50 and ED50.

Results

Effect of Dioxone on the Behaviour and Convulsant Action

Mice. A 1 mg./kg. dose of dioxone intraperitoneally produces only a slight increase in the animals' reactivity, especially revealed by behaviour with the investigator, particularly when attempting to catch the animal. The hyper-reactivity becomes more marked after doses of 3 mg./kg., but the spontaneous activity does not appear to increase. After 10 mg./kg., the spontaneous activity was often reduced, always accompanied by hyper-reactivity. Tremors, clonic convulsion and motor incoordination phenomena occur after the administration of 30 mg./kg. Higher doses provoke generalised tonic-clonic attacks with intervals of prostration and death of some animals.

Preliminary systematic studies of the effects on mice, made according to the method used in this laboratory (Maffii 1959), showed that the effects of dioxone could not be distinguished from those of leptazol at twice the dose.

The spontaneous motility of mice receiving 5 and 25 mg./kg. of dioxone and 10 and 50 mg./kg. doses of leptazol intraperitoneally was registered by a cage with a moving floor. No increase of the spontaneous activity in mice from the effect of subconvulsive doses of the two compounds was

observed during the 6 hr. after administration, the activity being reduced in some instances.

The tracings after the two treatments were not distinguishable.

The convulsant action in mice has been studied quantitatively, determining the ED50 effecting clonic and clonic-tonic attacks obtained through different routes of administration.

Table I compares the data obtained with leptazol, bemegride and dioxone and shows that dioxone is much more active than leptazol and slightly less active than bemegride. The ratios between this ED50 of dioxone and that of leptazol for different routes of administration, were found to vary between 1 : 1·7 and 1 : 2·25. The corresponding ratios

TABLE I

CONVULSANT ACTION IN MICE

Compound	Route of administration	No. of dose	Animals/ dose	ED50 (Fiducial limits: P = 0·05) Clonic seizures	ED50 (Fiducial limits: P = 0·05) Tonic seizures
Dioxone	i.v.	3	8	11·8 (9·7–14·3)	24·6 (19·7–30·7)
	i.p.	3	8	24·0 (21·7–26·6)	50·0 (45·5–55·0)
	oral	3	10	41·5 (29·0–59·3)	108 (84·5–138·2)
	s.c.	6	10	31·0 (26·9–35·6)	58·1 (52·8–63·9)
Leptazol	i.v.	3	8	23·0 (19·8–26·7)	42·0 (37·2–47·5)
	i.p.	5	10	54·0 (47·4–61·6)	92·0 (81·5–103·9)
	oral	3	10	91·0 (65·5–126·6)	—
	s.c.	8	10	62·0 (59·0–65·1)	101·0 (90·1–113·1)
Bemegride	i.v.	6	13	10·6 (10·1–11)	14·9 (13·3–16·6)
	s.c.	6	10	23·0 (21·1–25)	39·5 (36·4–42·8)

between the ED50 of bemegride and dioxone are 1 : 1·12 (i.v.) and 1 : 1·34 (s.c.). Increase in dosage causes a proportional increase in the duration and frequency of the convulsive attacks, while the latent period between administration and onset of the convulsion tends to be shortened.

Rats. Dioxone at 1 and 3 mg./kg. i.p. produces only slight reduction of the spontaneous activity. Tremors and some isolated clonus of the muscles of the limbs are observed with 10 mg./kg. Generalized clonic convulsions, repeating until death intervenes, occur with doses above 15–20 mg./kg. Clonic convulsions occurred in all animals at 30 mg./kg.; in 33 per cent they turned into tonic-clonic attacks. On oral administration, the first symptoms (tremors and muscular twitches) of the action of dioxone appeared 10 min. after a dose of 50 mg./kg. 60 mg./kg. produced clonic convulsions in 4 animals out of 9 and, in one, clonic-tonic attacks were followed by death. The duration of the single attacks increases regularly with repetition of the paroxysms.

The ED50 values for clonic seizures, as produced by different routes of administration, are gathered in Table II.

Rabbits and cats. In rabbits, intravenous administration of 5 mg./kg. of dioxone produces clonus of the masticatory muscles and some respiratory excitation immediately after injection. A generalized clonic attack was exceptionally rare. Higher doses (8–10 mg./kg.) led to fully clonic convulsions and, less frequently, to tonic-clonic attacks. All the animals survived after a period of prostration after the convulsive attack. In cats, intraperitoneal administration of 15 and 20 mg./kg. caused clonic and tonic-clonic convulsions preceded by a period of excitation and accompanied by salivation and sometimes vomiting. The animals survived the convulsive phenomena.

Dogs. Intraperitoneal administration. 5 mg./kg. of dioxone administered to dogs produced only slight muscular twitches and a small increase in respiratory frequency in one dog out of three; these effects disappeared after 50 min. In two experiments, 10 mg./kg. caused an increase in respiratory frequency and a few weak clonic contractions even 10 min. after the injection. An attack of vomiting occurred after 1 hr. in one animal. The animals appeared normal after 120 min. Respiratory excitation, salivation and vomiting appeared at a dosage of 20 mg./kg.

TABLE II

CONVULSANT ACTION OF DIOXONE AND BEMEGRIDE IN RATS

Compound	Route of administration	No. of doses	Animals/dose	ED50 mg./kg. (approximate) Clonic seizures
Dioxone	s.c.	4	10	16
	i.p.	3	15	15
	oral	3	10	55
Bemegride ..	s.c.	5	10	17

One dog out of two receiving this dose displayed generalised clonic convulsions 15 min. after administration, but both the animals appeared to be normal within 1 hr. of injection.

A 30 mg./kg. dose administered to several animals produced repeated clonic and tonic-clonic attacks, starting from 4 to 30 min. after injection.

Nevertheless, there was great variation in the susceptibility to convulsions. That the convulsive effects are linked to individual characteristics has been confirmed in cross-over tests in which the same animal received leptazol or dioxone. After several days the alternative drug was then given. The dogs displayed identical symptoms at the effective doses. Leptazol comparatively tested in some of the animals, displayed a series of symptoms identical with those observed after dioxone, but only at higher doses. Slight muscular twitches, increase of the respiratory frequency, salivation and attacks of vomiting were observed at doses of 10 and 20 mg./kg. Clonic convulsions were observed at 30–40 mg./kg. with a wide variation in incidence and degree. Repeated tonic-clonic attacks, accompanied by salivation and motor incoordination, and followed by prostration, were seen with 60 mg./kg. As with dioxone, the animals appeared to be normal within about 2 hr. after administration.

Oral administration. By this route dioxone also produces the characteristic convulsive symptoms. Two animals displayed clonic convulsions

with respiratory excitation and vomiting at 30 and 50 mg./kg. It has been observed that the latent period before the appearance of an attack varied from 35 to 60 min.

Persistence of the action and effects of successive administration. To determine the persistence of the effect, subconvulsive doses of dioxone (10 mg./kg.) and of leptazol (20 mg./kg.) were administered subcutaneously at 20, 40, 60, 90 and 120 min. Experiments on the same animal, with dioxone and leptazol, were made at 7 days' interval. Three of the 6 dogs used for this "cross-over" test were treated first with dioxone and secondly with leptazol. The results confirmed that the equivalent effective doses of dioxone and leptazol are in a ratio of 1:2 for dogs, as well as for mice and rats. The persistence of the effects, is about the same for both compounds and depends much upon the animals. For this reason it is difficult to establish the interval of time necessary between separate doses to avoid summation of effects. Greater responses were observed after the second administration with intervals of 20, 40, 60 and 90 min., and in some days even when the interval was prolonged to 2 hr.

Effects of Dioxone in Decerebrate Animals

The experiments were carried out on rabbits, cats and dogs. Dioxone and the other compounds, were administered intravenously at doses between 2·5 and 20 mg./kg. In rabbits, dioxone produces accentuation of the rigidity which may be followed by contractions of the muscle of limbs, isolated or grouped in deambulatory movements, and accompanied by respiratory stimulation. Only in 2 animals out of 10 were obvious convulsive attacks seen. The general picture of the changes produced by dioxone was substantially the same in cats except that movements of deambulation were usually absent. Also, in one rabbit out of 9, true convulsive phenomena were seen. This animal had received only 2·5 mg./kg. The reasons for these three erratic results are difficult to explain as the experimental conditions were the same and on autopsy the sections appeared to be complete and at the intercollicular level. Also in 2 out of 5 cats given bemegride (1–5 mg./kg. i.v.) we observed clonic seizures followed by death. As with dioxone, the animals which had convulsions were not those receiving the larger dose.

In the dogs neither dioxone nor bemegride (in doses from 2·5–7mg. /kg.) produced convulsions. Occasionally some tremors and increase in rigidity were seen. In 5 of 6 amimals both dioxone and bemegride produced marked stimulation of respiration.

Effects on Spinal Animals

In spinal rabbits and cats dioxone injected intravenously up to 20 mg./kg., produces intense clonus of the muscles with cranial innervation, but no effect on the limbs and trunk.

Antagonism by Anticonvulsant Agents toward the Convulsant and Lethal Effects of Dioxone

The action of some drugs noted for their anticonvulsant effect and their antagonism towards leptazol has been studied on the convulsive and

lethal results produced by dioxone. We considered trimethadione, meprobamate, phenobarbitone (leptazol antagonists) and diphenylhydantoin, which, has an anti-epileptic action, but which is inactive against leptazol-induced convulsions. Table III gives the 50 per cent protective doses (PD50) for the 4 drugs used against the lethal phenomena produced by 70 mg./kg. doses of dioxone intraperitoneally and 140 mg./kg. doses of leptazol. It would therefore appear that substances capable of antagonising the effects of leptazol also exert this action with dioxone. Diphenylhydantoin was found to be equally inactive in both instances.

Acute Toxicity

Table IV compares the LD50 values of dioxone, leptazol, and bemegride administered by different routes in various species. All produced death after tonic convulsions and generally within a short time of administration (from 20 min. i.v. to 3 hr. orally). Apart from the similarity in

TABLE III

ANTAGONISM BY DIFFERENT ANTICONVULSANT AGENTS TOWARDS LETHAL EFFECTS OF DIOXONE AND LEPTAZOL

Anti-convulsant	No. of doses	No. of mice/ dose	ED50 (protective) (70 mg./kg. dioxone)	No. of doses	No. of mice/ dose	ED50 (protective) (140 mg./kg. leptazol)
Trimethadione	5	6	269 mg./kg.	3	7	320 mg./kg.
Meprobamate	5	6	38 ,,	3	18	39 ,,
Phenobarbitone	4	6	~ 10 ,,	2	12	~ 14 ,,
Diphenylhydantoin	1	5	>200 ,,	1	5	>200 ,,

symptoms and death rate a quantitative difference among the three analeptics was evident, bemegride being the most toxic and leptazol the least. It may be observed, that in the rat the oral LD50 is relatively lower than in the mouse for all the compounds tested.

Subacute and Chronic Toxicity

Dogs. A preliminary test was made on 2 dogs, each of which received 5 mg./kg. of dioxone (1 per cent solution), daily intraperitoneally for a month without signs of toxicity. Haemochromocytometric tests, and prothrombin times before and after treatment, did not reveal any significant changes. A weekly examination of the urine did not disclose any pathological symptoms. The glycaemia and the Takata-Ara reaction, determined 15 and 30 days after treatment, were normal.

Two dogs received for 6 months oral doses of 30 mg./kg. of dioxone daily in 3 doses at 4 hr. intervals. Both animals survived. Alteration of character, sociability, appetite, or motor functions was not observed. In one dog, isolated clonus of the muscles of the head and the neck occurred three times during the 6 months. Being fully grown animals, their weights remained constant. Only occasional attacks of vomiting occurred during treatment 3 and 4 times respectively in the 6 months. No changes of a pathological nature were observed in the tests on urine and blood, made as described under "methods".

At autopsy, no gross pathological signs were observed in lungs, heart, spleen and kidneys. Liver was found slightly augmented in volume. Some slight subacute inflammation was found in the stomach and small intestine of both animals. Microscopically slight albuminous degeneration of the liver and kidneys of one dog were the only pathological findings.

Two dogs received 10 mg./kg. of dioxone twice daily subcutaneously for 6 months. The changes produced differed substantially in the two animals. In one dog repeated emesis was observed (53 days in 182),

TABLE IV

ACUTE TOXICITY IN MICE AND RATS

Compound	Animal	Route of administration	No. of doses	Animals/dose	LD50 mg./kg. (Fiducial limits)
Dioxone	mouse	i.v.	4	10	31·5 (26·7–39·9)
		i.p.	4	10	52·0 (45·7–59·3)
		oral	7	13	130 (106·6–158·6)
		s.c.	3	10	60·5 (54–67·7)
	rat	i.p.	4	10	31·6 (26·11–38·24)
		oral	5	10	70·5 (50·7–98)
		s.c.	4	8	39·4 (35·8–43·3)
Leptazol	mouse	i.v.	5	11	51·0 (45·65–56·96)
		i.p.	5	10	96 (88·8–104·6)
		oral	4	10	162 (135–194·4)
		s.c.	5	10	101 (93–109·5)
	rat	i.p.	4	10	70 (63·2–77·5)
		oral	4	10	140 (115·7–169·4)
		s.c.			100 (4)
Bemegride	mouse	i.v.	6	10	18·5 (15·9–21·4)
		s.c.	3	10	40·5 (36·1–45·3)
	rat	s.c.	4	10	30·5 (32·1–28·9)

together with anorexia and a moderate loss of hair, that appeared on the 35th day of treatment. The other dog did not show any peculiar symptoms. Vomiting occurred on 2 days in 183. Neither loss of hair, nor diminution in appetite was noted. Both animals kept a constant weight. At macro- and microscopic examination at the end of the 6 months no serious pathological signs were detected. Only signs of albuminous degeneration of some zones of hepatic parenchyma were observed in the first dog. The site of injection showed only minor signs of local irritation. The blood counts and analysis of urine and blood did not show any deterioration during the treatment, except for a sporadic appearance of a reducing substance in the urine of the first dog during the 2nd and 4th months.

Rats. Two groups of male rats received oral doses of 15 and 30 mg./kg. 6 days a week for 6 months. The compound was dissolved in distilled water, at 0·5 per cent for the lower dose and 1 per cent for the higher dose. The volume administered was 0·3 ml./100 g. weight. Few symptoms were observed during the period of treatment. On the 36th day one animal showed a small zone of alopecia that disappeared spontaneously within a week. One rat of the group treated with 30 mg./kg. showed three episodes of clonic seizures on the 71st, 124th and 141st day of treatment. No significant changes were observed in the test animals during and at the end of treatment, compared with the controls receiving only distilled water though the increase in weight was higher in the animals receiving dioxone. Complete blood counts of every animal did not show any significant changes from pretreatment levels.

The macroscopic examination and the weights of kidney, liver, spleen and adrenals did not differ from untreated animals. Histological examination showed albuminous degeneration of liver in 2 animals treated with 15 mg./kg., and in 4 animals treated with 30 mg./kg. Kidneys, spleen and heart did not show any sign of damage.

The same experiment was made over 6 months on groups of 10 animals each animal receiving dioxone intraperitoneally at daily doses of 10 and 25 mg./kg. Those receiving 25 mg./kg. were given the analeptic in two fractions at eight hourly intervals.

Dioxone was given in 0·416 per cent aqueous solution (pH = 4·8) and the injected volume was 0·3 ml./100 g. weight.

Rats receiving 10 mg./kg. did not show any unusual pathological symptoms except for a clonic attack in one animal during the last week of treatment. In the group receiving 25 mg./kg., tremors and muscular twitches were observed especially after the second daily half-dose. After the 23rd day of treatment clonic seizures were occasionally observed until the end of the treatment.

No animals died among those receiving up to 10 mg./kg. Seven out of 10 rats given 25 mg./kg. died at the 28th, 37th, 58th, 75th, 86th, 95th and 113th day respectively. All deaths occurred after the second daily half dose, during clonic seizures. Blood counts, made during and at the end of the experiment did not show any deviation from normal. At the end of the experiment the weights of animals receiving 10 mg./kg. were not significantly different from the controls given distilled water, and the three survivors of the group treated with 25 mg./kg. showed a normal increase of weight. Microscopic examination of the principal organs at the end of the treatment showed no sign of irritation in the peritoneal cavity. Kidneys and liver were augmented in volume in the animal receiving the higher dose.

Discussion

Dioxone is a new compound which exhibits interesting neurophysiological properties. It possesses a definite and noticeable convulsant action which is similar in many respects to that of leptazol and bemegride.

In mice and rats, dioxone produces clonic convulsions in sub-lethal doses but tonic seizures are induced by doses approaching the lethal ones as occurs with leptazol and bemegride. By comparing the ratios between the LD50 and ED50 of the three convulsant agents as shown in Table V it appears that the figures for LD50:ED50 ratios for tonic seizures are uniform both amongst the three analeptics and between different routes of administration, indicating that in animals given dioxone or the other two convulsant agents the tonic extensor phase of convulsions is related to severe toxic phenomena and mortality either produced by the convulsive status itself or—less likely—produced directly by the agents. Moreover as far as the LD50:ED50 ratios for clonic seizures are concerned a

TABLE V

RATIOS BETWEEN ACUTE TOXICITY AND CONVULSANT ACTION OF DIFFERENT AGENTS IN MICE

Compound	Route of administration	LD50 ED50 (clonic seiz.)	LD50 ED50 (tonic seiz.)
Dioxone	i.v.	2·66	1·28
	s.c.	1·93	1·03
Leptazol	i.v.	2·21	1·21
	s.c.	1·62	1·00
Bemegride	i.v.	1·76	1·24
	s.c.	1·76	1·02

quantitative difference has been found between dioxone and the other two drugs, which suggest that dioxone has a more specific activity than either bemegride or leptazol.

The ratio found with dioxone, by i.v. administration, is 2·66, a figure higher than any other known synthetic drug with similar properties. The convulsant activity of dioxone is also easily demonstrated in rabbits, cats and dogs.

In an attempt to investigate the origin of the convulsive phenomena and thereby approach the problem of site of action of dioxone, it can be concluded from the results obtained in spinal animals, that the new analeptic does not act on the spinal cord, since high doses do not produce convulsions or any other muscular activity in muscles with spinal-innervation.

In this respect dioxone—like bemegride and leptazol—differs from strychnine and also from nikethamide which possess a marked action on the spinal cord at convulsant dosage (Han and Schuk, 1956).

In decerebrate animals the effect of dioxone appeared less clear-cut. Although experiments were made on a relatively large number of animals the results were not so consistent as in spinal animals. In the dogs and in rabbits and cats, only minor motor phenomena were seen as well as an increase in the rigidity. In a few rabbits and cats we were able to produce true, generalised convulsions both clonic and tonic-clonic. We cannot explain at present the reason for these results on the basis of data available.

The convulsive effect of dioxone is counteracted by phenobarbitone, meprobamate and trimethadione, and this evidence further confirms its similarity with leptazol. As the seizures induced by dioxone may also be antagonised by the phenyl-4-alkyloxazindiones active against leptazol-induced convulsions (Maffii and Silvestrini 1961) and since the active doses of the anticonvulsant agents are about the same when dioxone and leptazol are used in isodynamic dosage, it seems reasonable to suppose that the antagonism between dioxone and anticonvulsants is functional rather than competitive.

Experimental evidence shows dioxone has a prompt and relatively long-lasting action, and is well absorbed when administered orally. Its acute toxicity is low especially if one considers the high level of activity of the compound, and chronic toxicity studies in rats and dogs did not reveal severe pathological features. This fact is particularly significant in considering that in the 6 month treatments, maximal subconvulsive doses were administered both daily and twice daily.

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