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Antipressor Effect of 1,4-Cyclohexandione on Blood Pressure of Hypertensive Dogs.*

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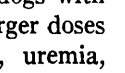
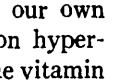
In a previous communication it was shown that some of the quinones are effective in lowering the blood pressure of hypertensive rats.[1] In the course of extending these observations to hypertensive dogs it was noted that, although some antipressor activity is possessed by the quinones, these chemicals were extremely toxic and their use sometimes resulted in the death of the treated dog.[2] It has recently been demonstrated that one of these quinones (xyloquinone) is effective in lowering the blood pressure of the dogs with "neurogenic" hypertension, but in larger doses produced hemolysis, tubular casts, uremia, and death.[3] Other work as well as our own has indicated an antipressor effect on hypertensive rats of the well-known quinone vitamin K (menadione).[4]

It was believed by us, however, that a further search should be made among compounds related to the quinones for antipressor agents with less toxicity. The quinones may, for our purpose, be regarded as highly reactive forms of unsaturated cyclic diketones. Accordingly, a search was begun among the carbonyl compounds (both aldehydes and ketones) for further antipressor agents.[2] It was quickly noticed that *mono*carbonyl compounds (aldehydes, ketones, and cyclic ketones) were inactive. Among the *di*carbonyl compounds (dialdehydes and diketones) certain of the shorter, straight-chain dialdehydes were able to reduce the pressure of both hypertensive rats and hypertensive dogs, but were extremely toxic to both animals. In this group belong glyoxal  and succinic dialdehyde  . The shorter chain compound was more active and more toxic than the longer chain dialdehyde. The 6-carbon analogue (adipic dialdehyde) was quite inactive. The straight-chain diketones were without exception inactive.

Inasmuch as the dialdehydes were active and the diketones were not, it seemed reasonable to suppose that the presence of two carbonyl groups was essential for activity, but that the effect depended upon the fact that the carbonyl groups in the quinones and in the aldehydes are more active than those present in the diketones. Inasmuch as cyclic diketones are somewhat more closely related to quinones than are open chain diketones and dialdehydes, it was considered desirable to investigate the activity of the cyclic diketones 1,2- and 1,4-cyclodexandione. Since 1,2-cyclohexandione is inactive in hypertensive rats as reported by us in a previous paper,[2] this compound was not investigated for its effect in dogs.

The *methods* previously described[1] for producing hypertension in the dog were again employed. In most experiments the hypertension was produced by the use of the Gold-

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TABLE I.
Responses of Hypertensive Dogs to Intramuscular Injections of 1,4-Cyclohexandione.

Dog No.	Duration of hypertension before treatment, months	Blood pressure before treatment, mm Hg	Daily dose of 1,4-cyclohexandione, mg/kg/day	Period treated, days	Blood pressure after treatment, mm Hg	Fall in blood pressure, mm Hg	Latency of response, days	Interval before return of blood pressure to hypertensive level, days
177	24	180/115	10	7	135/90	45/25	2	15
204	15	180/120	4-22	12	175/120	5/0	—	—
216	14	190/120	7	9	147/70	43/50	3	6
218	14	190/127	7	9	153/100	37/27	?	3
219	14	185/120	6-24	12	163/110	22/10	5	10
177	27	178/110	10	7	140/74	38/36	3	12
204	18	190/110	"	"	157/75	33/35	3	5
216	17	181/107	"	"	148/77	33/30	3	8
218	17	182/110	"	"	162/80	20/30	3	11
219	17	188/106	"	"	160/82	28/24	6	11
239*	1	170/102	"	"	155/80	15/22	2	3
240†	2	180/108	"	"	144/74	36/34	3	—
Avg		183/113			153/86	30/27	3.6	7.4

* Dog 239 was made hypertensive by a Goldblatt clamp on left renal artery and cellophane wrapping of the right kidney.

† Dog 240 was made hypertensive by the injection of croton oil into left renal artery and subsequent application of a Goldblatt clamp to the right renal artery.

blatt clamp, but in one dog an emulsion of croton oil was twice injected into the left renal artery and subsequently the right renal artery was clamped, and in still another dog the right kidney was wrapped in cellophane according to the method of Page and the left kidney was clamped. The blood pressure was measured by the indirect auscultatory method using a sphygmomanometer cuff and mercury manometer. The dog was considered hypertensive when the high blood pressure was maintained for one month or more. The drug, which is quite soluble, was dissolved in distilled water and injected intramuscularly into the lumbar muscles. Blood pressures were taken daily, but were omitted on some holidays. The dose of 1,4-cyclohexandione varied from 4-24 mg per kg per day. The blood pressures recorded in Table I are the separate medians of the systolic and the diastolic pressure for the 5 days preceding the treatment, and for the period during which the antipressor response was maintained.

Results. The results are shown in Table I. The experiments have been divided into 2 series. In the first series 5 hypertensive dogs were injected with varying doses of 1,4-cyclohexandione, and in the second the same 5 dogs with the addition of 2 other hypertensive dogs were each given 10 mg per kg of body weight daily for 7 days. Although one of the five dogs failed to respond and another gave only a moderate antipressor response in the first series, all the dogs including these 2 responded well in the second series in which the optimal dose was used.

Evidently the compound, 1,4-cyclohexandione, had an antipressor effect in these hypertensive dogs. It should be noted that the blood pressure did not fall immediately after the administration of the compound, but that a latent period of 3 to 7 days intervened in all cases. Moreover, after injections were stopped, a similar but somewhat longer interval elapsed before the blood pressure returned to the pre-treatment level.

The intramuscular injections in hypertensive dogs were apparently painless and produced no gross evidence of local or general toxic reactions. Large doses, many times the optimal therapeutic dose, injected into normal rats and normal dogs had no effect on the blood pressure, and gave no evidence of toxicity in either species.

The administration of 10 mg per kilo of the same compound to 2 normal dogs for 6 days did not affect their blood pressure.

Discussion of Results. Our previous results on hypertensive rats have shown that 1,2-cyclohexandione is inactive, but that 1,4-cyclohexandione possesses moderate antipressor activity. Although 1,4-cyclohexandione is much less active than some of the quinones previously investigated, it is so much less toxic to rats and dogs that it offers greater promise as an antipressor agent than any of the other 34 compounds we have studied thus far.

The mode of action of all these antipressor agents is still obscure. The antipressor activity of the dicarbonyl compounds declined in the order, *p*-quinones, *o*-quinones, dialdehydes, cyclic diketones, straight-chain diketones, and their toxicity decreased in the same order. The cyclic diketones are the first group of compounds in this series which are not too toxic for use.

Summary. Daily intramuscular injections for one week of the diketone, 1,4-cyclohexandione, into hypertensive dogs resulted in a moderate reduction of blood pressure. In the dosage thus far employed there has been no gross evidence of local or general toxic effect.

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An Improved Method for Testing of Bacteriostatic Agents Using Virulent Human Type Tubercle Bacilli.*

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The demonstration by Feldman and his coworkers[1-3] and others[4,5] of the therapeutic effect of various compounds on experimental tuberculosis of guinea pigs increases the necessity for a rapid and accurate *in vitro* method for testing the relative growth inhibitory powers of compounds on *M. tuberculosis var. hominis.*

Several investigators[5-15] have used virulent human tubercle bacilli for this purpose, employing a variety of media both liquid and solid. The use of complex solid or liquid media is unsatisfactory because of the possible presence of antagonists; agar when added to a synthetic medium is in itself slightly inhibitory.[16] The use of liquid synthetic media has previously required relatively large volumes of medium in flasks, to provide sufficient surface growth, inoculated on the surface with flakes of pellicle growth. The results are usu-

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