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Effect of Dibenamine on Renal Function.*

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Clinical interest[1-3] in Dibenamine (N,N-dibenzyl-β-chloroethylamine) has made a full understanding of its toxicity essential. Previous reports from his laboratory[4,5] indicate two major types of toxicity: (1) central nervous system stimulation, particularly when the drug is administered rapidly by the intravenous route, and (2) local tissue damage after subcutaneous or intramuscular administration. Before Dibenamine was made available for clinical trial, a histological study of the organs of rats receiving maximum tolerated or lethal doses for protracted periods was undertaken;[6] this study failed to reveal any renal damage even in animals dying from chronic Dibenamine administration.

The recent report of Ogden[7] that Dibenamine produced renal failure in one dog has questioned the safety of this agent. The present work was undertaken in an effort to evaluate more exactly the effect of Dibenamine on renal function.

Methods. Experiments were carried out on 5 female dogs weighing between 10 and 15 kg which were trained to lie quietly with only loose restraint during the collection periods. Glomerular filtration was determined by creatinine clearance and renal plasma flow by sodium p-aminohippurate (PAH) clearance. Approximately constant plasma levels of creatinine and PAH were maintained by the continuous infusion (about 8 ml/min) of 5% glucose solution containing creatinine and PAH. The plasma creatinine level was maintained at about 10 mg % while the PAH level was usually below 2 mg %. Bladder urine was collected through a rubber mushroom catheter, and at the end of each 10-minute collection period the bladder was washed with distilled water. Blood samples were taken at the midpoint of each urine collection period, by means of a syringe moistened with heparin solution. Creatinine was determined by the alkaline-picrate method[8] and PAH by a modification[9] of the method of Bratton and Marshall.[10]

Dibenamine‡ (20 mg/kg) was administered intravenously once each week for 6 weeks. The animals were divided into two groups. Group A (3 dogs) received Dibenamine in 100 ml of 0.9% NaCl solution over a period of 45 to 60 minutes. Group B (2 dogs) received the entire dose of Dibenamine in one to 5 minutes, after preliminary sedation with 15 mg/kg pentothal sodium intravenously to reduce the severity of the convulsions which

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‡ The Dibenamine employed in these experiments was supplied by Givaudan-Delawanna, Inc., as a 5% solution in acidified alcohol-propylene glycol in sterile ampuls prepared for clinical investigation.

TABLE I.
Renal Function in Dogs Before and After Dibenamine Administration.

Dogs		Group A						Group B			
		1 (12.3 kg)		2 (13.4 kg)		3 (14.2 kg)		4 (13.6 kg)		5 (10.0 kg)	
		G.F.R. ml/min.	R.P.F. ml/min.	G.F.R. ml/min.	R.P.F. ml/min.	G.F.R. ml/min.	R.P.F. ml/min.	G.F.R. ml/min.	R.P.F. ml/min.	G.F.R. ml/min.	R.P.F. ml/min.
Control		46.6	130.3	61.7	181.9	56.2	168.6	57.9	163.6	42.2	121.5
,,		45.6	125.3	60.4	178.9	57.0	166.9	57.6	160.3	41.8	122.3
,,		45.9	126.0	60.5	180.5	58.2	172.1	57.7	162.2	41.4	121.9
After Dibenamine											
Successive weekly inj.*	1st	46.1	110.8	58.8	162.6	56.2	165.8	58.2	150.2	42.8	144.0
	2nd	45.0	121.5	64.0	191.8	57.6	172.3	57.5	152.0	44.3	125.5
	3rd	47.6	126.5	61.7	185.2	57.0	178.4	57.2	144.2	41.0	126.4
	4th	47.6	130.0	63.2	170.0	58.8	160.4	57.7	194.5	42.4	127.8
	5th	47.8	130.6	60.1	179.2	58.8	158.2	56.9	217.2	44.4	130.9
	6th	(42.0) †	(86.2)	(62.4)	(144.6)	(56.5)	(124.7)	(57.1)	(162.9)	(44.5)	(118.2)
		47.7	127.7	60.6	188.5	56.9	155.7	57.0	200.8	44.4	132.5

* Renal function tests performed one week after each injection.

† Figures in parentheses determined at height of adrenergic blockade, 1½ hours after 6th Dibenamine injection. Note return to normal one week later.

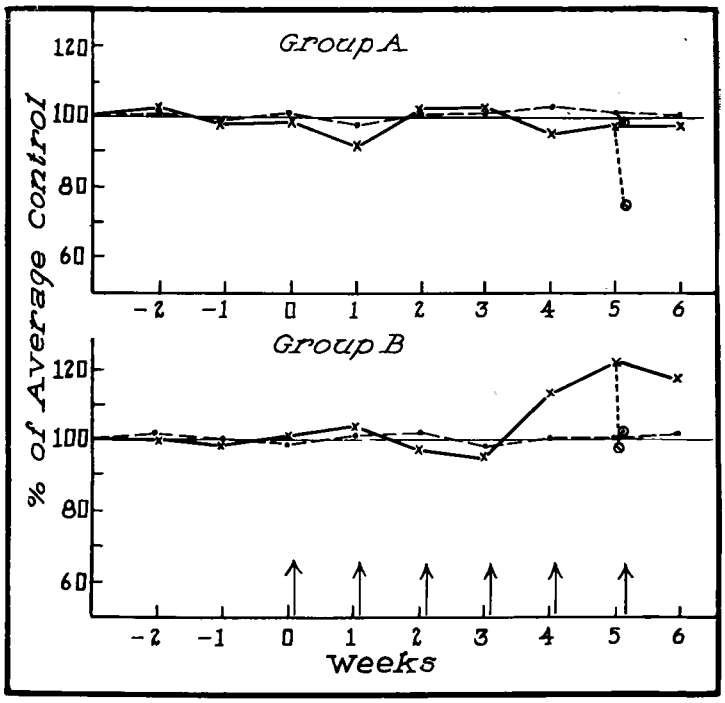

FIG. 1.

Mean glomerular filtration (•——•) and renal plasma flow (x——x) plotted as per cent of average control values for each group of dogs. Dibenamine (20 mg/kg) administered intravenously at each arrow. Circled points indicate determinations made 1½ hours after Dibenamine administration.

result from the rapid administration of such large doses of the drug. The vein was flushed with 0.9% NaCl solution at the end of the injection to prevent thrombophlebitis. Renal function was redetermined one week after *each* Dibenamine injection, and also once in each animal during the height of the Dibenamine action (1½ hours after the 6th weekly injection). Determinations of the mean systemic arterial pressure were made on several animals before and after Dibenamine administration by the use of a capillary mercury manometer and direct femoral artery puncture.

Results. The results of all clearance tests are shown in Table I and Fig. 1. In Fig. 1, mean rates of glomerular filtration and renal plasma flow are plotted as percentage of the average of three control determinations for each animal.

No persistent alterations in glomerular filtration or renal plasma flow occurred in any of the animals, with the exception of a moderate increase in renal plasma flow in one dog receiving rapid injections of Dibenamine. This increased plasma flow was accompanied by a decrease in the filtration fraction. Its significance is difficult to evaluate.

Clearance determinations made during the height of the adrenergic blocking action of Dibenamine showed a consistent reduction in renal plasma flow, which was essentially the same in the two groups; but no reduction in the glomerular filtration rate occurred. In no case did the reduction in renal plasma flow persist until the following test, *i.e.*, after wearing off of the adrenergic blocking action of the Dibenamine.

Determinations of mean systemic arterial pressure indicated about a 30% decrease 2 hours after Dibenamine administration (*e.g.*, 145 to 100 mm Hg).

With the large doses of Dibenamine employed, side-effects were not uncommon. Animals receiving the drug by slow infusion always developed hyperpnea and showed other

evidence of mild central nervous system. stimulation. They salivated profusely and usually defecated. Vomiting occurred at least once in each animal, but was never observed later than the third injection in any series. As expected, the immediate toxic reactions were severe when the drug was administered in a period of 5 minutes or less. Vomiting and defecation occurred regularly and clonic convulsive movements developed after each rapid injection in spite of the barbiturate sedation. These animals were prostrated for a period of 24 to 48 hours, but appeared normal before the next weekly test. None of the animals showed any significant weight loss during the course of the experiment.

Discussion. A careful reevaluation of the renal toxicity of Dibenamine was made necessary by the work of Ogden[7] who reported that 2 weekly intravenous administrations of 20 mg/kg Dibenamine caused persistent marked reductions in renal plasma flow and glomerular filtration in one dog. He also described histological evidence of tubular degeneration. A subsequent report by the same author[11] discussed 2 additional animals given more numerous injections of Dibenamine with successively less evidence of renal damage. Ogden reported symptoms of shock in his animals immediately after the Dibenamine injections. Although injection rates were not given, it seemed possible that the alleged renal damage was related to the too rapid injection of the drug, particularly in the first animal studied. The much increased toxicity of Dibenamine after rapid intravenous injection has been repeatedly reported and warned against.[2,4,5]

Because of the possibility that a failure to observe precautions regarding the rate of Dibenamine infusion was responsible for the reported renal damage, dogs in the present experiments were divided into two groups. Group A received the drug by slow infusion with minimal side-effects. Group B received the entire dose of drug within 5 minutes for three injections, and when this procedure failed to produce persistent changes in renal function the remaining injections were completed within 1 minute each. The latter procedure provided the maximum stress possible with this dose of Dibenamine, but it failed to produce detectable renal damage.

These observations on renal function provide confirmation by more sensitive tests of the conclusion from histological observations[6] that even massive doses of Dibenamine do not produce significant renal pathology.

In the absence of a detailed report on the experiments of Ogden, we are unable to offer any explanation for the discrepancy between his results and our own. The fact that he observed decreasing renal damage in successive animals suggests that some factor in the experimental procedure may have been responsible for his findings, but our data indicate that the rate of injection is not a major factor.

The consistent decrease in renal plasma flow but not glomular filtration during the adrenergic blocking action of Dibenamine deserves special mention. This effect was observed almost equally after both slow and rapid injection. The decrease in renal plasma flow can probably be accounted for on the basis of vasodilatation in vascular beds other than renal, and the consequent lowering of the systemic arterial pressure. The decrease in blood pressure observed by direct femoral artery puncture in several animals was greater than that usually observed in anesthetized animals[4,5] or normal recumbent humans.[1,2] It is perhaps correlated with the initially high mean systemic arterial pressure in dogs and suggests that sympathetic vasomotor tone is an important factor in maintaining their blood pressure.

The increased filtration fraction (avg. 21.7%) after Dibenamine indicates a differential effect on afferent and efferent arterioles. Dibenamine has not been shown to have any direct vasconstrictor action, and the similarity of the changes observed with fast and slow injections of the drug suggests that any renal vasodilation was probably due to the adrenergic blockade *per se*. If the Dibenamine induced changes in filtration fraction are explained entirely on the basis of a release of vasomotor tone (adrenergic blockade), it

[11] Ogden, E., Report at Meeting of Fed. of Am. Soc. for Exp. Biol., 1948.

must be assumed that the tonic neurogenic constriction of afferent arterioles is greater than that of efferent arterioles. This interpretation is a variance with the conclusion of Chasis, *et al.*[12] that the efferent arteriolar bed is the major site of vasomotor regulation in the human kidney, and the conclusion of Hiatt[13] that the renal circulation of the dogs has little or no vasomotor tone.

It is clear that our present knowledge of the complex mechanism controlling renal blood flow is inadequate to provide a definitive explanation of the mechanism by which Dibenamine increases the renal filtration fraction. It is possible that in our experiments the lowered blood pressure and renal blood flow after Dibenamine induce a sufficient production of renin to alter the picture of pure vasomotor blockade. Additional experimental work is required to elucidate the mechanism of the Dibenamine induced increase in filtration fraction. Clarification of this problem may also provide a better understanding of renal vasomotor regulation.

Summary. 1. Six weekly intravenous injections of Dibenamine (20 mg/kg) failed to produce any detectable renal damage as measured by creatinine and PAH clearances in 5 trained, unanesthetized female dogs, even when the entire dose was injected within one minute so as to produce maximum toxic side-effects including prostration for 24 to 48 hours.

2. During maximal sympathetic blockade by Dibenamine, renal plasma flow was reduced 10 to 34% (average 21%), probably on the basis of reduced systemic arterial pressure. However, the filtration fraction was sufficiently elevated to provide unaltered glomerular filtration. These alterations never persisted beyond the period of active adrenergic blockade.

3. We have been completely unable to substantiate the observations of Ogden[7] on the renal toxicity of Dibenamine or to find any renal basis for his warning regarding its use. Even large doses of Dibenamine administered very rapidly appear to have no significant reno-toxic action.

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Enhancement of Penetration of Penicillin into Inflamed and Normal Mucous Membrane by Hyaluronidase.

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Duran-Reynals[1] first described the presence in testicular extract of a factor which is capable of facilitating the spread of vaccine virus in the skin of rabbits. Since then, this factor has been shown to be identical with hyaluronidase.[2] The enzyme has been found capable of spreading such diverse substances as vaccine virus, dyes, india ink, rabies virus, hemoglobin, diphtheria toxin, glucose and methemoglobin. It appeared to the authors that it would be of interest to determine whether penetration of penicillin into a mucous membrane might also be enhanced by hyaluronidase particularly since therapeutic failure with penicillin is sometimes ascribed to the inability of the antibiotic to reach deep-seated foci of infection. Our studies were carried out as follows:

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