

# OBSERVATIONS ON DIURETIC ASSAY METHODS USING RAT AND DOG

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## INTRODUCTION

A number of proven measures and drugs capable of producing diuresis, in congestive heart failure and other clinical conditions associated with edema, have been available for many years but still the need for more effective, less toxic and more convenient remedies is self evident.

The screening of diuretics for clinical utility is a matter of practical importance. Promising laboratory results often are not supported by subsequent clinical trials. Such discrepancies may arise from the fact that the final clinical evaluation is determined in the patient with congestive heart failure, a condition which is extremely difficult to achieve in animals. It may also be due to species difference.

A method using rats for estimation of antidiuretic potency was described by Burn in 1931. This method or a modification of it has been used for diuretic assays by most of the subsequent workers. In 1943, Lipchitz *et al.* described a method suitable for diuretic assay using several commonly used diuretics. This method by itself is a modification of Burn's method. Since 1943, most of the workers have used the method of Lipschitz *et al.* with some modifications. Chen in 1956 described a new method for diuretic assay.

Workers who used dog as an experimental animal for assay of diuretics varied in the technical details much more than those who used rats. Dogs used were either anesthetized or unanesthetized. Mostly unanesthetized trained female dogs were used. Sometimes the female dogs were prepared beforehand by some pre-operative procedure to allow easy catheterization. Animals were either hydrated with saline, or with distilled water, or not hydrated at all.

Since there are so many different ways in which various drugs have been evaluated for their diuretic property, we thought it would be interesting to compare few commonly used diuretics in an assay method using both dogs and rats, and thus compare the suitability of animals. It was also our inten-

tion to standardize a method for evaluating new drugs for their diuretic activity. Our experience and results obtained with urea, theophylline, acetazolamide and mersalyl, in rats and dogs, on a comparative basis is presented here.

#### MATERIALS AND METHODS

All experiments were done on adult male mongrel dogs and male albino rats.

##### *Dogs :*

In all 20 dogs were used. Five dogs were used each for mersalyl, theophylline, acetazolamide and urea. Weight of dogs varied from 9—18 kg.

All dogs were anesthetized by Dial 70 mg./kg., half of it being given intravenously and half intraperitoneally. Animals were hydrated by using 20 ml./kg. of 0.9% saline given intravenously at the rate of 10 ml./kg./hour.

Urinary bladder of the animals was exposed by a median incision over the lower part of abdomen. Both ureters were identified, cleared and cannulated about an inch above the urinary bladder; using polyethylene tubing of suitable diameter and length. Urine was collected and volume recorded every 15 minutes.

Control observations were done for about one and one half to two hours after the saline drip was over. The drug was then injected and urine volume recorded for a further period of five hours.

A detailed statistical analysis of results in dogs was done. Control readings were taken as concomitant variable. Analysis of co-variance was done since it was felt that results after administration of the drug might have been affected by the concomitant variable (control observations).

##### *Rats :*

Adult male albino rats weighing between 150-350 gms. were used in groups of four. Food and water was left available till the experiment started. During the experiment which lasted for five hours, no food or water was allowed. Each time at least one group of rats received urea, and others received theophylline, acetazolamide or mersalyl. Ten groups of rats were crossed over to receive another during next experiment with a minimum rest period of four days.

Each rat was primed by administering 25 ml./kg. of 0.9% saline intraperitoneally. Drug administered was dissolved in the saline injected for priming the animal.

Each group of four rats was kept in a metabolic cage and urine collected free from feces. Urine volume was recorded at the end of five hours. With mersalyl 24 hour urine volume was taken into account, and control urea volume was also recorded for 24 hours when mersalyl was used.

Ten groups of rats were used each with mersalyl, acetazolamide, theophylline and urea. Five or 24 hour urine volume was expressed as percentage of initial hydration. Urea was taken as control and results were expressed as percentage increase over control (urea) run simultaneously.

*Dosage of drugs :*

Dosage of drugs was same for both rats and dogs, except in case of mersalyl. All drugs were injected by intraperitoneal route in rats and by intravenous route in dogs. Urea was used in the dose of 10 mg./kg., while sodium salt of acetazolamide was used in the dose of 5 mg./kg. Dose of mersalyl used was 6.6 mg./kg. in dogs and 15 mg./kg. in rats.

RESULTS

All the results of experiments in rats are given in Table I.

TABLE I

*Results in ten groups of rats.*

Percentage increase over control (urea) value after administration of drugs. Figures represent five hour figures for theophylline and 24 hour figures for Mersalyl.

	Theophylline	Acetazolamide	Mersalyl
	20	192	140
	37	191	310
	70	138	217
	61	207	153
	15	39	224
	0	74	219
	41	218	334
	15	57	245
	2	58	271
	3	20	447
Mean	26.4	119.4	256.0
S. E.	±7.8	±24.5	±20.4

Table II shows the average control 15 minute urine volume and average 15 minute urine volume for five hours following administration of drugs, in dogs, which was taken into account in the analysis.

TABLE II

		Urea Drug 1	Theophylline Drug 2	Acetazola- mide Drug 3	Mersalyl Drug 4	Total for all Drugs.
Dog 1	x	0.87	1.13	0.80	4.47	
	y	2.12	2.28	2.78	7.38	
Dog 2	x	2.53	1.60	2.66	2.31	
	y	3.27	5.72	4.63	5.94	
Dog 3	x	2.30	3.47	2.20	4.40	
	y	4.35	6.06	5.10	11.06	
Dog 4	x	2.57	2.30	1.88	3.80	
	y	5.28	3.03	3.13	13.48	
Dog 5	x	4.13	3.52	2.48	2.60	
	y	5.34	5.20	3.56	7.45	
Total for each drug	x	12.40	12.02	10.02	17.58	52.02
	y	20.36	22.29	19.20	45.31	107.16

Upper figure in each square represents mean urine volume per 15 minutes during control period which was treated as concomitant variable x.

Lower figure in each square represents mean urine volume per 15 minutes during five hours after administration of the drug (y).

*In dogs :*

The average rate of excretion of urine per 15 minutes during control period was treated as concomitant variable and the mean rate of excretion of urine per 15 minutes after the drug was administered, was treated as the response which was useful in assessing the efficacy of the drugs.

Analysis of co-variance was performed as given in Table III and it was found that the effect due to concomitant was significant, at 5% level of significance. It was also found out that the effect due to different drugs was significantly different again at 5% level of significance.

TABLE III  
*Analysis of covariance.*

Source	d.f.	x <sup>2</sup>	xy	y <sup>2</sup>
Between drugs	3	6.24	23.14	92.44
Error	16	16.24	18.35	61.69
Total	19	22.48	41.49	154.13

The effect of any drug may be affected by the various factors as shown in the following set up.

$$E(y_j) = \mu + \delta_j + \beta(x_j - \bar{x}) \dots \dots \dots (1)$$

$E(y_j)$  is the expected value of  $y_j$  which stands for the effect of  $j$ th drug;  $j =$  Drug 1,2,3,4.  $\mu =$  general mean,  $\delta_j =$  Effect of  $j$ th drug,  $\beta =$  concomitant coefficient, the departure of which from zero will indicate that the control readings should be taken into account while analysing for the differential effects of the drugs. From (i) we get,

$$\hat{\delta}_j = (\bar{y}_j - \bar{y}) - \hat{\beta}(x_j - \bar{x}) \dots \dots \dots (2)$$

$$\hat{\beta} = 1.13$$

Error sum of squares = 40.96 with 15 degrees of freedom.  
(for testing significance)

$$\hat{S}^2 = \frac{\text{Error sum of squares}}{\text{Degrees of freedom}} = 2.73$$

(a) to test  $\beta = 0$ ;  $F_{1,15} = 7.59^*$

(b) to test  $\delta_1 = \delta_2 = \delta_3 = \delta_4$ ;  $F_{3,15} = 4.47^*$

(c) to test various comparisons of drugs, taken two at a time,  
 $\delta_1 = -1.15$ ,  $\delta_2 = -0.68$ ,  $\delta_3 = -0.84$ ,  $\delta_4 = 2.67$ .

The variance of treatment is,

$$\frac{\sum (\bar{x}_i - \bar{x})^2}{r} + \frac{(\bar{x}_i - \bar{x})^2}{E_{xx}}$$

for testing,  $\delta_1 - \delta_2, t = 0.45, \delta_1 - \delta_3, t = 2.29, \delta_1 - \delta_4, t = 3.38^*$   
 $\delta_2 - \delta_3, t = 0.15, \delta_2 - \delta_4, t = 2.94^*, \delta_3 - \delta_4, t = 2.9^*$ , each comparison is with 15 degrees of freedom.

In comparing the effect of different drugs taken two at a time it was found that the effect due to mersalyl differed significantly (P-0.05) from the effects due to urea, theophylline or acetazolamide while the latter three drugs did not differ significantly amongst themselves.

#### Results in Rats :

All the results of experiments in rats are given in Table I. With theophylline the best estimate of true mean increase over control (urea) from a sample of ten groups was  $26.4 \pm 7.8$ . With acetazolamide the best estimate of true mean increase over control, from a sample of ten groups was  $119.4 \pm 24.5$ . With Mersalyl the best estimate of true mean rise over control from a sample of ten groups was  $256.0 \pm 20.4$ .

#### DISCUSSION

As diuretics are employed clinically in the treatment of edema, it would seem to be most important to demonstrate effectiveness in presence of additional electrolyte and water, at least in animals. Excess water and salt are given in animals to simulate edema. Since water itself acts as a diuretic<sup>12</sup> normal saline should be used to hydrate the animal. Animals can be hydrated by oral, rectal or intravenous route. Control and test observations in dogs were done in this study, only after the hydration was over, since it was found in preliminary experiments that urine volume varied directly as the rate of hydration.

#### Dog as an experimental animal :

Most of the workers have used unanesthetized trained female dogs<sup>4,5,6,7,8,9</sup> while some<sup>4,5</sup> have used anesthetized dogs for diuretic assays. Animal's response may vary on different days due to change in temperature, humidity, environment, diet, etc. To eliminate most of these factors, it may be preferable to use anesthetized animal.

Maloney<sup>10</sup> used three different drugs in the same anesthetized dog. In this study only one drug was given to one dog. All the drugs used in this study maintained a fair diuresis till at least for three hours. Moreover, it was

\*Values marked with asterisk are significant at 5 per cent level.

found that after using a diuretic in one dog, even another potent diuretic produced very little action.<sup>13</sup> Test period after administration of drug varied with different workers. Fulton<sup>5</sup> observed urine volume for 4 hours, Lipschitz *et al.* for 5-7 hours, Farah *et al.* for 1½ to 2 hours, Kuttus *et al.* for 24 hours and Little<sup>4</sup> for 5 hours. Observation period should be at least five hours after administration of the drug. During the sixth hour, with the drugs used in this study, viz., mersalyl, acetazolamide, theophylline and urea, urine volume returned to practically normal levels<sup>14</sup>.

Individual variation was seen to a great extent with acetazolamide and theophylline. Response was more constant with mersalyl and urea. In this study five dogs are used for each of the four drugs tested. At five per cent level of significance action of mersalyl differed from that of either acetazolamide, theophylline or urea; while the latter three drugs did not differ significantly among themselves.

The method used in this study was essentially that described by Lipschitz *et al.* differing from it in minor details. Rats were used in groups of four instead of eight, also rats were not starved for any length of time before the experiment. Lipschitz *et al.*, Little<sup>4</sup>, Von Arman<sup>15</sup>, and Chen<sup>3</sup> starved rats for varying periods of time. McColl<sup>16</sup> did not starve rats.

Most of the workers<sup>2,3,4,15</sup> used oral route for hydrating the animal. McColl<sup>16</sup> was the only one to use intraperitoneal route. In this study intraperitoneal, instead of oral route was used for following reasons.

- (a) It does away with irregular absorption through gastrointestinal tract.
- (b) Reduces handling of an animal to minimum.
- (c) Some of the drugs have to be administered parenterally in which case they can be given together with saline.
- (d) It compares well with the intravenous route of hydration in dogs, used in this study.

Test period was found to be sufficient when taken for five hours with urea, theophylline, and acetazolamide. But with mersalyl, it was necessary to observe 24 hour urine volume since very little diuresis occurs during first five hours. Period of observation for the controls also has to extend for 24 hours, when mersalyl is used. Mersalyl was used in the dosage of 15 mg./kg. and not 6.6 mg./kg. as used in dogs. This is the dose of mersalyl found to be most suitable in rats by Lipschitz *et al.*<sup>2</sup>.

Results expressed in this study are on the same pattern as that used by Lipschitz *et al.* viz. percentage increase (of percentage urine volume of initial hydration) over control group. Urea was taken as control. Variation between different groups was more with acetazolamide than with mersalyl, while it was maximum with theophylline. Drugs arranged in order of

ascending potency are : theophylline, acetazolamide and mersalyl. This is in confirmation with the results obtained by other workers.

*Comparisons of rat and dog as an experimental animal for diuretic assay :*

Rat as an experimental animal for diuretic assays gives more consistent results, since as a result of using animals in a group, biological variation gets reduced significantly. It is possible to use different doses of the same drug in different groups, together with control groups, and thus obtain very useful data within the space of a few experiments only.

Rat, can and has been used for accurate bioassays of new diuretics. Outstanding advantages of rat method are, it is economical, simple, reliable, more consistent and only small amounts of drugs are sufficient.

Both Von Arman<sup>15</sup> and Lipschitz<sup>7</sup> found that rats gave much more consistent results than dogs.

But as seen in this study, dog compares favourably well with the rat as far as testing or screening of new diuretic drugs is concerned, since within a few experiments only, one can have some information about whether the drug acts as a diuretic or not, and also about its potency. Another advantage of using a dog is that simultaneous investigations on the effect of the drug on renal hemodynamics, and on constituents of urine can be done, which may lead to mechanism of, or site of action of the drug.

It would be uneconomical to do accurate bioassay of diuretics in dogs since there is so much of variation and hence one would have to employ a large series.

It would be advisable to use in dog, a new diuretic which has been tried in rat and other lower animals, before trying it clinically. Modell, using diuretics in dogs, rats and patients with congestive heart failure, observed that the results in dog tended to resemble those in man much more closely than those in rats.

Finally to quote Lipschitz<sup>7</sup> "The figures for diuretic effects in dogs are more scattered, although obtained from the same individual, than in different groups of rats, and it appears that the rat assay method for diuretics is the method of choice. Nevertheless sequence of diuretics concerning their potency was found to be the same on the dog as on the rat. This fact seems important because it allows us to draw conclusions from the results gained with the rat assay method to effects on higher animals, and even to therapeutic doses for the patient." The same sequence was seen when the activities of diuretics in common use as found by this method were compared with the average therapeutic dose employed in patients.



## SUMMARY

(1) Mersalyl, acetazolamide, theophylline and urea were tested for diuretic activity in rats and dogs.

(2) A detailed statistical analysis of the results in dogs was performed.

(3) Action of mersalyl in dog was found to be significantly greater than that of urea, theophylline, and acetazolamide.

(4) Detailed technique for diuretic assays is described.

(5) A comparison of dog and rat as an experimental animal for diuretic assays is presented.

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