

# Blood pH and Plasma Levels of *d*-Tubocurarine

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The effect of changes in blood pH on the distribution of *d*-tubocurarine within the plasma was investigated in 12 animals. Using a modified Latin square design each dog was studied for 1 hour under conditions of normal blood pH, respiratory acidosis, and respiratory alkalosis. Circulatory depression was prevented by the infusion of 1,1-dimethyl-1,4-phenylpiperazinium (DMPP). Plasma levels of *d*-tubocurarine were measured spectrophotometrically at 3, 5, 10, 20, 30, and 60 minute intervals following the intravenous injection of 0.3 mg./kg. An analysis of variance indicates no significant difference between plasma concentrations of *d*-tubocurarine at various pH levels and suggests that changes in blood pH in the physiologic range have little effect on the plasma distribution of *d*-tubocurarine.

THE INFLUENCE of changes in blood pH on the plasma level of *d*-tubocurarine has been the subject of two recent publications.<sup>1,2</sup> In these studies an average increase of almost 100 per cent in plasma levels of *d*-tubocurarine was reported to be associated with a reduction of blood pH to 7.2. Significantly lower plasma concentrations of *d*-tubocurarine (70 per cent decrease) resulted from its intravenous injection at blood pH 7.6. These findings are at variance with normal plasma levels of *d*-tubocurarine observed by us in three dogs made severely acidotic (pH 6.90–7.04) for a study of the blood-brain barrier to *d*-tubocurarine.<sup>3</sup> A possible explanation for this discrepancy may be in the utilization by Utting and by Baraka of less sensitive analytic techniques, or alternatively, the result of precipitous falls of blood pressure reported in their experiments.<sup>1</sup> In the following study a constant effort was made to maintain blood pressure within normal limits, and plasma con-

centrations of *d*-tubocurarine were chemically analyzed by a spectrophotometric method sensitive to  $\pm 0.1$   $\mu\text{g./ml.}$ <sup>4</sup> The analytic technique utilized in all studies measures *d*-tubocurarine both in the bound and unbound states.

## Method

Four healthy dogs (average weight 18 kg.) were each anesthetized on three occasions with intravenous sodium pentobarbital, 30 mg./kg. A minimum time interval of two weeks was allowed between studies in an individual animal. Following endotracheal intubation, respiration was maintained on room air with a Harvard Pump Respirator. Using a modified Latin square experimental design, each animal was studied at three different levels of blood pH: normal, acidotic, and alkalotic. Respiratory acidosis (mean pH 6.98) was produced through the addition of 10 per cent carbon dioxide in oxygen to the inhaled atmosphere. Respiratory alkalosis (mean pH 7.74) was produced by hyperventilation. Arterial blood pH was determined at frequent intervals with a Beckman physiologic pH meter, and intra-arterial blood pressure was constantly monitored and recorded on an Offner Dynograph.

Following establishment of a steady pH level (similar pH measurements 10–15 minutes apart) *d*-tubocurarine 0.3 mg./kg. was administered intravenously and arterial samples withdrawn at 3, 5, 10, 20, 30 and 60 minute intervals for determination of plasma *d*-tubocurarine. All measurements were carried out in duplicate, each sample requiring 5 ml. plasma. A concerted effort was made to maintain the animal's blood pressure at pre-injection levels. This was satisfactorily accomplished in all animals by intermittent infusions of 1,1-dimethyl-1,4-phenylpiperazinium (DMPP), 0.2 mg./ml.\* Blood pH was again measured 15, 30, and 60 minutes following the injection of *d*-tubocurarine.

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\* DMPP supplied by Parke-Davis and Co.

TABLE 1. Plasma Levels of *d*-Tubocurarine Following Intravenous Injection 0.3 mg./kg. to Normal, Acidotic, and Alkalotic Dogs\*

Animal	Expt.	pH Range	Plasma <i>d</i> -Tubocurarine ( $\mu\text{g./ml.}$ )					
			3 Min.	5 Min.	10 Min.	20 Min.	30 Min.	60 Min.
820 (18 kg.)	7	6.94-7.05	2.6	2.6	2.0	1.5	1.2	0.7
	1	7.43-7.47	2.5	1.7	1.3	0.9	0.4	0.2
	4	7.66-7.76	2.3	1.9	1.5	1.1	0.7	—
991 (23 kg.)	5	6.80-7.17	3.5	3.3	1.7	1.0	0.8	0.6
	8	7.33-7.46	3.4	2.7	1.9	1.3	0.9	0.7
	3	7.76-7.80	2.9	2.4	1.7	1.0	0.8	0.5
794 (16 kg.)	2	6.84-7.04	2.8	2.4	1.8	1.2	0.9	0.6
	9	7.36-7.51	2.3	2.0	1.9	1.2	0.8	0.2
	6	7.65-7.80	2.7	2.1	1.3	1.1	0.7	0.2
239 (15 kg.)	10	6.93-7.03	3.4	2.2	1.7	1.1	0.9	0.4
	11	7.37-7.45	—	2.1	1.5	1.1	0.9	0.7
	12	7.70-7.80	2.9	2.3	1.7	1.3	1.1	0.7

\* Mean values of duplicate measurements.

In two additional experiments arterial *pH* was rapidly varied from an acidotic to an alkalotic and back to an acidotic state. These changes were accomplished by having the animal inhale 10 per cent carbon dioxide until a steady state of acidosis was reached (*pH* 7.07), and then suddenly removing the inhaled carbon dioxide while simultaneously hyperventilating the animal with room air. Following 20 minutes of the alkalotic state (*pH* 7.69), arterial *pH* was again lowered (*pH* 7.22) by adding 10 per cent carbon dioxide to the inhaled atmosphere. Blood pressure was maintained throughout at steady levels by intermittent infusion of DMPP. As in the earlier studies *d*-tubocurarine 0.3 mg./kg. was given in a single intravenous injection and arterial samples withdrawn at prescribed intervals and analyzed for plasma *d*-tubocurarine.

### Results

Results obtained in 12 experiments with four dogs may be found in table 1. Graphic representation of these data is shown in figure 1. Rapid redistribution of *d*-tubocurarine follows within the first 10 minutes of its intravenous injection initially resulting in a wide variation in plasma concentrations. Statistical analysis of the data was therefore limited to the 10 to 60 minute period representing the most stable part of the distribution curve. The

response variable utilized in the analysis was the sum of the four plasma levels measured during this latter period.

Since treatment order appeared to be an important effect, a covariance analysis was utilized to adjust the responses and eliminate order effect. The adjustments for each animal were +0.42 for the first experiment, +0.29 for the second experiment, and -0.71 for the third experiment. The treatment means, after adjustment for order effect, were 4.63, 3.80, and 4.02 for acid, neutral, and alkaline *pH*. An analysis of variance based on the adjusted data is shown in table 2. The 95 per cent confidence interval estimate for the true mean difference in responses to acidosis and alkalosis was computed to be  $0.61 \pm 1.06$  or (-0.45, 1.67). Thus no significant treatment effects were observed.

In table 3 are shown plasma levels of *d*-tubocurarine obtained in two animals by rapidly varying blood *pH* from the acidotic to the alkalotic state and back to the acidotic state. The plasma concentrations of *d*-tubocurarine thus measured may be compared to control values for the same animals.

### Discussion

Lack of significant variations in the plasma level of *d*-tubocurarine at various levels of blood *pH* was as expected. Kalow<sup>5</sup> has as-

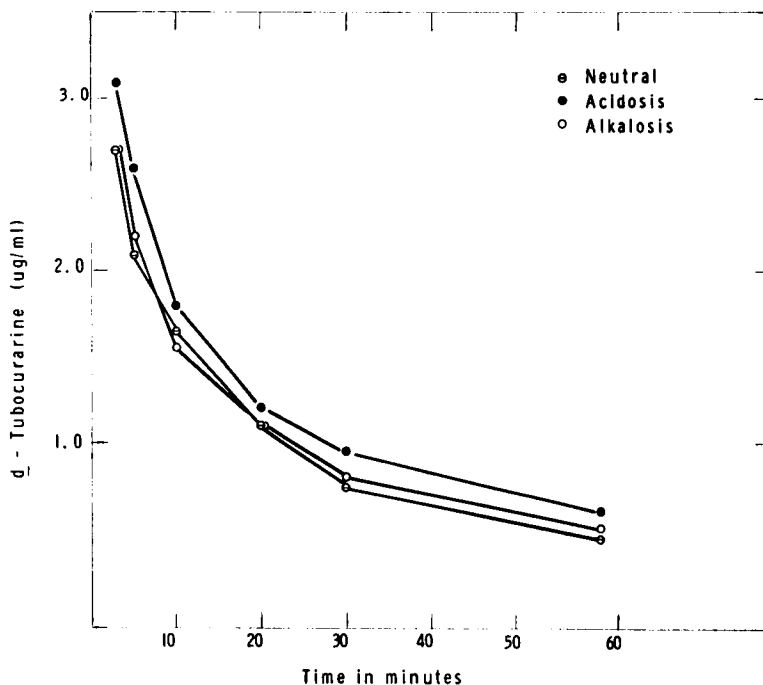


FIG. 1. Plasma levels of *d*-tubocurarine following the intravenous injection of 0.3 mg./kg. in the normal, acidotic, and alkalotic state. Each point represents the mean of four animals. (Statistical analysis of these data is shown in table 2.)

signed *pK*'s of 8.1 and 9.1 to the hydroxyl groups of *d*-tubocurarine, and the quaternary ammonium groups must be considered as fully ionized. Katz *et al.*,<sup>6</sup> utilizing data obtained by Albert,<sup>7</sup> have calculated that *pH* changes of 0.3 to 0.4 units in the physiologic range could only produce a 1 to 10 per cent change in the ionization of these hydroxyl groups. It is thus unlikely that physiologic variations in plasma *pH* could significantly influence distribution of *d*-tubocurarine through minor changes in ionization. Furthermore, in the *pH* ranges under consideration (*pH* 7.0-7.8), *in-vitro* studies have indicated that an increased binding of *d*-tubocurarine to plasma protein occurs with an increase in *pH*. Maximal binding is attained at *pH* 8.2. At yet higher *pH* the binding of *d*-tubocurarine to plasma protein sharply decreases.<sup>8</sup> It thus follows that physiologic ranges of acidosis would decrease the binding of *d*-tubocurarine to plasma protein and thus not interfere with its redistribution out of the plasma compartment.

It should be stated that the above considerations are independent of any direct action that *pH* or *P*<sub>CO<sub>2</sub></sub> may exert on neuromuscular transmission, or of any additional muscle blocking effect these variables may produce on the ad-

dition of *d*-tubocurarine. Katz and Wolf<sup>9</sup> have shown that the twitch response of the adductor pollicis muscle to supramaximal ulnar nerve stimulation can be increased by hyperventilation alone and decreased by breathing 10 per cent dioxide. The depression of twitch response which followed the injection of *d*-tubocurarine was reduced by hyperventilation and increased by 10 per cent carbon dioxide. A discussion of these phenomena is beyond the scope of this paper.

In the dog, hypotension is customary following the intravenous injection of *d*-tubocurarine and this phenomenon was observed in all animals. In previous studies, norepinephrine has been shown to be relatively ineffective in reversing severe degrees of hypotension. On the other hand, DMPP, a ganglionic stimulating

TABLE 2. Analysis of Variance

Source of Variation	<i>dF</i>	Sum of Squares	Mean Square	<i>F</i> Ratio	<i>P</i> Value
Dogs	3	0.4100	0.1366	0.47	0.70
Treatments	2	1.4836	0.7418	2.55	0.20
Error	4*	1.1628	0.2907		
Total	9*	3.0564			

\* Two degrees of freedom used in the adjustment for order effect.

TABLE 3. Plasma Levels of *d*-Tubocurarine Following Intravenous Injection 0.3 mg./kg.\*

	3 Min.		5 Min.		10 Min.		20 Min.		30 Min.		60 Min.	
	<i>d</i> -Tbc. ( $\mu$ g./ml.)	pH	<i>d</i> -Tbc. ( $\mu$ g./ml.)	<i>d</i> -Tbc. ( $\mu$ g./ml.)	pH	<i>d</i> -Tbc. ( $\mu$ g./ml.)	pH	<i>d</i> -Tbc. ( $\mu$ g./ml.)	pH	<i>d</i> -Tbc. ( $\mu$ g./ml.)	pH	
Control												
Dog 820	2.5	7.47	1.7	1.3	—	0.9	7.43	0.4	7.45	0.2	7.46	
Dog 991	3.4	7.46	2.7	1.9	—	1.3	7.33	0.9	7.40	0.7	7.40	
Experimental												
Dog 820	—	7.06	2.7	1.6	7.02	1.3	7.62	—	7.74	0.7	7.18	
Dog 991	3.3	7.08	2.7	1.8	7.02	0.9	7.74	0.6	7.63	0.5	7.26	

Blood pH rapidly varied from acidotic to alkalotic to acidotic state.

\* Mean values of duplicate measurements.

agent, proved effective in supporting blood pressure under a wide variety of stress situations.<sup>3</sup> (Possible interaction between DMPP and *d*-tubocurarine cannot be ruled out at the present time, although the significance of this factor is not pertinent to the results observed since the same combination of drugs was used in both acidotic and alkalotic animals.) A marked tendency to hypotension was noted in each instance following the injection of *d*-tubocurarine to the severely acidotic dog. Under similar conditions in the normal or alkalotic animal only temporary support of blood pressure was required. Since moderate to severe hypotension is likely associated with a slower circulation and redistribution of the *d*-tubocurarine, one should anticipate a reduced rate of loss of *d*-tubocurarine from the plasma compartment. This could then account for the higher plasma levels of *d*-tubocurarine in the hypotensive acidotic animals as reported by Utting.<sup>1</sup> By carefully maintaining all dogs in a normotensive state, we were able to prevent this abnormal distribution.

### Summary

Plasma concentrations of *d*-tubocurarine were determined in four healthy dogs following the intravenous injection of 0.3 mg./kg. under conditions of normal blood pH, respiratory acidosis, and respiratory alkalosis. Twelve experiments were performed using a modified Latin square design with each dog serving as its own control. Statistical analysis of the data indicated no significant differences between plasma concentrations of *d*-tubocurarine

at various pH ranges in groups of animals. Studies in 2 animals in whom blood pH was rapidly altered from the acidotic to the alkalotic to the acidotic state showed no deviation from the normal plasma distribution curve for *d*-tubocurarine. The above findings suggest that changes in blood pH in the physiologic range have little effect upon the plasma distribution of *d*-tubocurarine.

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