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Stephen Callender Schimpff
Yale University

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Stephen Callender Schimpff

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CONTENTS

Introduction

Animal experiments (1,2,3) and clinical observations (4,5,6) suggest that the amount of digitalis required to produce toxicity is influenced by alterations in acid-base equilibrium. For example, infants in heart failure generally require a high dosage of digitalis, in mg. per Kg., to produce therapeutic or toxic effects (7,8,9,10). Infants with heart failure secondary to left-to-right cardiac shunts have been observed to have a respiratory acidemia and mild hypoxemia (11). These alterations in acid-base equilibrium are possibly asscciated with the infants' increased digitalis tolerance (11).

This thesis will report on animal experiments in which a digitalis tolerance test was used to assess the effects of respiratory and metabolic acidemia and hypoxemia in modifying the amount of digitalis required to produce toxicity.

REVIEW OF THE LITERATURE

A. History - Digitalis Toxicity

Today digitalis is used primarily for heart failure and cardiac arrhythmias; such was not always the case. The toxic properties of digitalis were once considered therapeutic properties: Galen and Hippocrates used squill as an irritant, emetic, and expectorant. West African savages used a tree root called Waybo (ouabaio) as an arrow tip poison. During the 14th century, digitalis was used externally for wounds and ulcers and internally for epilepsy. Later it was used as an expectorant (Gerrarde, 1590) and emetic (Parkinson, 1640) (12).

In 1785 William Withering popularized the use of digitalis for dropsy in his book "An Account of the Foxglove and Some of its Medicinal Uses" (13). He thought that digitalis improved dropsy by a direct action on the kidney; yet, he also noted that digitalis had a "power over the motion of the heart to a degree yet unobserved in any other medication, and this power may be converted to salutory ends". Withering wrote an excellent description of the effects of overdosage: "The foxglove when given in very large and frequent doses occasions sickness, vomiting, purging, giddiness, confused vision, objects appearing green and yellow, increased secretion of urine with frequent motions to part with it and sometimes inability to retain it; slow pulse, even as slow as 35 a minute, cold sweats, convulsions, syncope, and death."

Not heeding Withering's warnings regarding the potential danger of digitalis and thus the need for individualization of dose,

practioners of the day often "overdosed" their patients to the degree that the "cure" seemed worse than the disease (12). So often were toxic manifestations observed that Ferriar (14), 1799, a well known and highly respected English physician wrote, "If I am acquainted with any undubitable fact in medical practice, it is the power of digitalis in retarding and weakening the action of the heart and arteries."

Ferriar based his opinions on an analysis of 24 patients* given digitalis compared with ten patients given cream of tartar. None of the 24 were "cured" with digitalis but six of the ten were "cured" with tartar and with fewer "toxic" problems. As a consequence, he concludes "the result appears not highly in favor of digitalis, yet it is valuable...and safe, by attending to Dr. Withering's caution" (14).

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* In reviewing Ferriar's 24 cases, I find (1) the ages range from 9 to 58 years, (2) the symptoms range from "anasarca" alone to a combination of orthopnea, chest "oppression", and decreased sleep, and (3) no attempt was made to describe the severity or duration of the symptoms. His disregard for placing each patient in an appropriate diagnostic category, rather than just dropsy, invalidates his comparisons between digitalis and tartar and his conclusions on the adverse effects of digitalis. However, his were the accepted beliefs of the day.

Although others, notably Hamilton (15), attempted to revive digitalis to its rightful place in therapeutics, the often observed toxicities and the prestige of Ferriar relegated it to the pharmacists

shelf until early in the twentieth century. It was not until Wenchebach (12) recommended its use for heart failure in 1910 that digitalis really came into its own.

Yet to this time, the close relationship between toxic and therapeutic dosage levels lends an air of mystery to the "digitalization" of a patient.

B. Principle Therapeutic Uses of Digitalis Today

The primary therapeutic action of digitalis is its positive inotropic effect on the heart. In patients with heart failure, the inotropic effect leads to an increased cardiac output as a consequence of increased myocardial contractility. This basic property, increased contractility, also occurs in the non-failing heart (16, 17,18,19).

The other therapeutic property of digitalis is its negative chronotropic effect. In the failing heart, digitalis lowers the heart rate as a consequence of a reflex response via vagal stimulation secondary to the increased cardiac output. Atropinization, however, does not inhibit this effect, which suggests that there is also a direct effect of digitalis on the sinoatrial node (10).

In atrial fibrillation, digitalis acts via increased vagal tone and by a direct action to increase the effective refractory period of the atrioventricular node. This leads to conduction of fewer atrial impulses and hence a slower ventricular rate.

"Toxic" doses of digitalis lead to arrhythmias secondary to the so-called increased "automaticity" of the conducting fibers.

This effect is related, probably, to changes in the membrane potential and to an increased rate of diastolic depolarization (20). In adults, premature ventricular contractions are often the first sign of cardiac toxicity followed by bigeminy, ventricular tachycardia, fibrillation, and death. In infants, supraventricular arrhythmias are usually the first toxicities to be observed, followed later by the changes seen in adults (7,21,22). There are some known predisposing factors to cardiac toxicity which include renal failure, diabetic ketoacidosis, excessive use of diuretics leading to potassium depletion, myocarditis, and perhaps cor pulmonale (10,23). Premature infants and neonatal infants are very prone to toxic manifestations at low dosage (24 ,25); whereas, infants from 1 month to 12 months of age generally require about twice the dosage of digitalis, on a milligram per kilogram basis, than do adults to produce either therapeutic or toxic effects (8,9,10, 7).

C. Metabolism and Subcellular Location of Digitalis

An understanding of the metabolism and cellular location of digitalis in both normal and disease states might help in explaining variations in digitalis tolerance. Recently, the metabolism and excretion of digitalis has undergone much study but the cellular localization remains unclear (20,26,27,28,29,30).

Tritium labelled digoxin (H^3-digoxin) has been administered to patients and its metabolism and excretion observed. It's organ location has been studied at post-mortem examination (31). When given intravenously, H^3-digoxin is found to have a half life of about 36 hours with 90% excreted in the urine within seven days. The remaining

ten per cent is found in the stool. Almost all of the digitalis found at the heart is unmetabolized H^3-digoxin suggesting that it, and not a metabolite, is the active principle. The myocardial concentration is regularly about 30X the serum concentration under all conditions; this suggests the potential value of a serum digitalis assay. In renal failure, excretion time is prolonged, and blood and tissue levels are correspondingly higher longer. Doherty (31) observed that patients with renal failure often tolerate "normal" maintenance doses of digoxin but that their body potassium and digitalis stool excretion are generally elevated.

Radioactive digitoxin (C^{14}-digitoxin) has a half life of about seven days, and residual radioactivity is found in the urine at 40 days. Digitoxin, unlike digoxin, enters the enterohepatic pathway where it remains until slow metabolism to more polar metabolites, including digoxin, allows its excretion via the kidney (26).

Renal insufficiency increases the half life of both H^3-digoxin and C^{14}-digitoxin, but liver disease has not been shown to slow excretion of either compound. There is no available data comparing half life times under conditions of hypoxemia, acidemia, or electrolyte imbalance.

Very little is known about the metabolism and excretion of acetyl strophanthidin. Lown (32) observed its rapid onset of action (from 30 seconds to 12 minutes) and its rapid subsidence of effects. Two hours after toxic manifestations occur (ventricular tachycardia), the same full dosage must be given to reproduce toxicity. This suggests a process of rapid metabolism to inactive compounds, rapid

excretion, or inactivation by storage in body tissues for later excretion.

The cellular localization of digitalis is unclear (28). H^3-ovabain is said to be found near the A bands with light microscopy and autoradiography (27). Electron microscopy and autoradiography have supposedly shown digitalis at the A-I junction (28) and at the sarcotubular system (27). Page (29) feels that there have been no adequate investigations that prove there is any intracellular digitalis. Lack of accurate data and conflicting reports have added impetus to the proliferation of theories (see below) on the mechanism of action of digitalis. There is no evidence at the present time that various disease states or metabolic imbalances lead to alterations in sub-cellular location as an explanation of variations in digitalis tolerance.

D. Tissue and Cellular Actions of Digitalis

There are at least three theories which attempt to describe how digitalis exerts its primary therapeutic effect of increased myocardial contractility. These theories revolve about three possible sites of action: (1). the myofibrillar apparatus, (2). the cell membrane and its electrical potential and ionic gradients, and (3). the system that "couples" the action potential to the contractile mechanism. An understanding of these possible mechanisms of action may help to explain the onset of toxic manifestations under varying conditions.

The evidence for the role of digitalis at the myofilaments is indirect. Isolated actomycin preparations are said to exhibit "improved" spiraling in the presence of digitalis and digitalis is said to inhibit myosin ATPase (17). These may be significant observations if the

reports that digitalis is located at the A-I junction are substantiated.

Unlike the myofilament studies, the action of digitalis at the cell membrane, especially the erythrocyte cell membrane, is reasonably well documented. At all digitalis dosage ranges, cellular potassium declines secondary to active transport inhibition. Post mortem analyses of digitalized hearts show significant decreases of intracellular potassium (33). Coronary sinus catheterization of dogs has shown a prompt and marked potassium loss from myocardial cells, and arterial blood samples indicate that considerable additional potassium ions are mobilized from as yet undefined body tissues (33). The amount of potassium lost from the myocardial cell is in the range of 15% and is due to decreased potassium influx across the cell membrane by inhibition of an ATPase and, hence, inhibition of "active transport" (33). Experiments with erythrocytes also show potassium loss with a sodium and water gain in the presence of digitalis (29,34). With in vitro experiments, increased extracellular potassium in the presence of digitalis leads to intracellular potassium loss and sodium gain. This suggests that the high extracellular potassium blocks the site of action of the digitalis. On the other hand, if extracellular potassium is very low, potassium escapes and sodium enters without the stimulus of digitalis. This also suggests that some critical level of extracellular potassium is necessary for the sodium-potassium active transport system and may lead toward an explanation of why increased serum potassium inhibits digitalis toxicity (29,35).

Areskog (36) has shown that acetyl strophenthiden leads to not only a rise in coronary sinus potassium but also to a rise in hydrogen ions, suggesting a myocardial cellular loss of both potassium and hydrogen.

At present, it appears that digitalis, by inhibiting myocardial ATPase, blocks myocardial cell uptake of potassium which changes the transmembrane potential. Because of the sodium-potassium imbalance in Purkinje tissue secondary to digitalis, the resting membrane potential declines and the rate of diastolic depolarization increases. This produces the observed increase in "automaticity" (or pacemaker activity) of the conduction tissue and, hence, the various cardiac arrhythmias seen with digitalis "overdosage" (28).

Intravenous infusion of potassium chloride is often used to modify digitalis toxicity, but Williams, et al., (37) have shown that potassium does not decrease inotropic activity. Acetyl strophanthidin was infused into adult dogs until severe arrhythmias occurred; then potassium was infused until the heart returned to sinus rhythm. No change was observed in the degree of myocardial contractility as the potassium was infused; in fact, the return to sinus rhythm allowed for more infusion of digitalis with subsequent rise in contractility.

It seems clear, therefore, that changes in the sodium-potassium gradient are probably associated with the production of digitalis toxicities and that intravenous infusion of potassium modifies tolerance. Metabolic derangements of the body may lead to variations in digitalis toxicity via modifications of the transmembrane ionic concentrations.

Finally, there is the widely held theory that digitalis exerts its action on the calcium-dependent "coupling" mechanism. The action potential at the cell membrane is followed in 2-3 milliseconds by a slight muscle relaxation (latency relaxation), then 4-5 milliseconds later, by muscle contraction. If calcium is removed from

the bathing medium, there is no contraction following the action potential (28); thus, calcium is said to "couple" electrical excitation to myofibril contraction. With each contraction, calcium ions diffuse into the cell, and within limits, increased extracellular calcium leads to greater contractility (28,38). Electron microscopy shows that the cell membrane (sarcolemma) invaginates and produces the "intracellular" sarcotubular apparatus. Calcium is found extracellularly and has been shown by autoradiography with Ca^{45} to be bound near the sarcotubular apparatus (28). This means that the action potential and the external bathing media come in close proximity to the contractile proteins. Actin and myosin depend on ATP for their contractility, and myosin ATPase depends on calcium for activation (28).

The "coupling" theory at present suggests that the action potential leads to intracellular movement of calcium or sarcotubular release of bound calcium. The calcium diffuses to the myofilaments and activates myosin ATPase, then contraction occurs.

There is speculation that digitalis, also shown by some to be located at the sarcotubular apparatus (27), leads to release of additional calcium and hence increased contractility. This assumption is based on the concept that since digitalis inhibits the ATPase required for sodium-potassium active transport, it may also inhibit the ATPase system required to bind calcium to the sarcotubular apparatus during relaxation.

Although this is an interesting theory in regard to the inotropic action of digitalis, it does little to explain the mechanism of toxicity production; however, calcium metabolism must not be

forgotten in an overall assessment of digitalis action. It is well known that acidosis leads to an increase in serum calcium (39) and significantly elevated serum calcium levels may lead to arrhythmias (23).

E. Digitalis Tolerance and the Metabolic Alterations of Heart Failure.

Little is known about the effects of metabolic changes on digitalis tolerance, yet most patients with heart failure have significant acid-base and blood gas alterations. Left heart failure and pulmonary edema in adults often lead to arterial oxygen desaturation with a compensatory hyperventilation and resultant respiratory alkalosis (23). Adults with heart failure secondary to cor pulmonale often have a respiratory acidosis with low oxygen saturation. Recently, Talner, et al., demonstrated that infants in heart failure secondary to congenital left-to-right shunts had a respiratory acidemia and hypoxemia. These infants had the following average arterial blood values: pH 7.33; PCO_2 51.4mm Hg; PO_2 55.7mm Hg; oxygen saturation 79.4%; and serum potassium 5.0m Eq. per liter.

Observations of adult patients suggest that hypoxemia and, perhaps, hypercapnia lead to a decrease in digitalis tolerance. Corazza and Pastor (5) found that ten per cent of all serious arrhythmias in patients with cor pulmonale were secondary to digitalis toxicity. Dreifus (4) observed an increased incidence of arrhythmias in geriatric patients, some of whom developed respiratory acidosis while on maintenance digitalis. Adams (40) claims that the response to digitalis in patients with cor pulmonale depends on the degree of pulmonary insufficiency and that improved pulmonary function results in an increased

digitalis effect. Burgland, et al., (41) used cardiac catheterization to study patients with cor pulmonale taking digitalis. Administration of oxygen during exercise resulted in a decreased ventricular filling pressure but no change in cardiac output. In the only available adequately controlled human experiment, Baum (6) gave 1.2 mg acetyl strophanthidin to each of 29 patients with chronic lung disease while they underwent cardiac catheterization. The electrocardiogram was monitored and some patients showed evidence of toxicity. The eight patients that developed toxicity had a significantly lower PO_2 than the 21 patients who did not show evidence of toxicity. No correlation was found between PCO_2, pH, or potassium alone and the tolerance to digitalis. It appears, therefore, that hypoxemia tends toward decreasing digitalis tolerance.

Three animal experiments have been done which suggest a relationship between blood pH, potassium, and digitalis tolerance. When Shafer (1) produced metabolic acidemia in adult dogs by infusing ammonium chloride, he found an increased serum potassium and an increased digitalis tolerance. Bliss (2) produced similar results by infusing hydrochloric acid into adult dogs. Shafer and Bliss suggested that the lowered pH lead to an increased serum potassium which increased digitalis tolerance. Talso (3) found a decreased tolerance in metabolic alkalosis. Finally, Areskog (36) suggested a relationship between an increased inotropic effect of acetyl strophanthidin during moderate respiratory acidosis.

A few reports exist on the effect of pH and PCO_2 on digitalis action in isolated heart tissue preparations. Using Purkinje tissue

and measuring transmembrane potentials, Karis, et al. (42), and Heeht and Hutter (43) have shown that pH changes lead to membrane potential changes. When digitalis was added to the media, respiratory or metabolic acidosis produced an increased automaticity where none existed with digitalis alone or with pH changes alone. If the PCO_2 and bicarbonate were altered to elevate the PCO_2 or lower bicarbonate, while maintaining pH constant, then increases in automaticity, in the presence of digitalis were still observed.

F. Digitalis Tolerance Test

In 1953, Lown, et al. (32) introduced the acetyl strophanthidin tolerance test which has since been used to determine the state of digitalization in patients and to study the effects of drugs and metabolic changes on the quantity of digitalis required to produce toxicity in animals and man. Acetyl strophanthidin is the most rapid acting digitalis-like compound known. Seventy per cent of the peak effect is observed within five minutes after intravenous administration. Moreover, the drug's action dissipates so rapidly that it is possible to "redigitalize" at 2 hour intervals without evidence of accumulation. Acetyl strophanthidin's action is additive to other cardiac glycosides in the body. Thus, determining the amount of acetyl strophenthidin required to produce a standard toxic end point in a patient gives an indication of the patient's previous degree of digitalization. This test, according to Lown, is helpful in determining whether an observed arrhythmia is due to digitalis already in the body or due to a need for additional digitalis.

This same tolerance test has been used repeatedly in the

past 14 years to compare the quantity of digitalis required to produce a toxicity (ventricular tachycardia is commonly used as the toxic end point) in an experimental situation as compared to a baseline control in the same or similar animal (1,2,3,32,44).

The digitalis tolerance test is the experimental method used in this thesis.

MATERIALS AND METHODS

A. Selection and Preparation of Experimental Population

Forty-five puppies from nine litters of unknown breeds were anesthetized with pentobarbital, 30 mg/Kg, intraperitoneally. A tracheostomy was performed and the puppy was ventilated with a volume-controlled small animal respirator. Both femoral arteries were cannulated for blood sampling and blood pressure monitoring and both femoral veins were cannulated for infusions and drug administration. Rectal temperature was monitored and a heated operating table was used to maintain a stable body temperature at 37 ± 1°C.

Once the animal's condition was judged to be stable, base-line values of arterial blood pH, PCO_2 and PO_2, serum potassium, EKG (lead 2), blood pressure and heart rate were obtained. Only those puppies whose initial studies were normal were kept in the experiment. The animals that failed to maintain a stable blood pressure, rectal temperature, blood gas, and pH values (except where these were intentionally altered) were excluded. Thirty-three animals fulfilled these requirements; data on their respective age, sex and weight are listed in Table 1. Arterial blood samples were analyzed at 37°C for pH with an Astrup pH electrode, for PO_2 with a modified Clark electrode, and for PCO_2 with a Severinghaus electrode. Oxygen saturation was calculated from PO_2 and pH with the nomogram by Rossing and Cain (45). All values are expressed at 37°C. Serum potassium was determined by flame photometry. The volume of blood lost through sampling (5-10% of total blood volume) was replaced with an approximately equivalent amount of saline solution.

B. Determination of Digitalis Tolerance

Digitalis "tolerance" was defined as the total amount of acetyl strophanthidin (A.S.) in µg AS/Kgs required to produce a specific toxicity as observed on the electrocardiogram: four consecutive premature ventricular complexes (2,18). A.S. was administered intravenously in an initial dose of 40 µg/kg and followed by 10 ug/Kg every five minutes thereafter until digitalis tolerance was achieved. The EKG was monitored continuously and frequent recordings were made until the four consecutive PVCs appeared.

C. Procedure

1. Controls

Every other puppy served as a control. After baseline studies were obtained, these animals remained on room air ventilation and their digitalis tolerance was determined. During the time required to determine tolerance (from 30 to 90 minutes) the pH was checked every 15-25 minutes and blood gases every 40-50 minutes. Within one minute of the appearance of toxicity, repeat studies were done for blood pH, PCO_2 and PO_2 and serum potassium.

2. Respiratory Acidemia

Respiratory acidemia was produced in seven puppies from four litters by administering a gas mixture of 15% CO_2 in air (18% O_2) via the respirator. Once a stable pH and PCO_2 had been achieved (within 30-45 minutes), new blood samples were taken for pH, PCO_2, PO_2, and potassium ("acidemic" sample). Digitalis tolerance was then studied as in the controls while a stable state of acidemia was maintained.

3. Respiratory Acidemia and Hypoxemia

This was produced in five puppies from three litters with a gas mixture of 10% CO_2, 10% O_2 and 80% N_2. The procedure was identical to the one described for control and respiratory acidemia studies.

4. Metabolic Acidemia

Two puppies from one litter were studied. Metabolic acidemia was induced with an intravenous infusion of 3-4 ml of 1N hydrochloric acid at a rate of 0.034-0.068 ml/min. (via a Harvard infusion pump). Digitalis tolerance was determined as in the controls while the acidemia was maintained.

5. Hypoxemia

Two puppies were studied using a gas mixture of 10% O_2 and 90% N_2. The pH and PCO_2 remained at control levels. Digitalis tolerance was determined as in the other litters.

For ready comparison, Table III lists the average pH, PCO_2, PO_2 and oxygen saturation for the control, acidemia, and hypoxemia groups. Individual puppy blood gas and pH values are listed in Table II; digitalis tolerance is listed in Table I for each puppy.

Metabolic Acidemia in Cats - Effects on Digitalis Tolerance

In addition to the 33 puppies, four adult cats of unknown breed, weighing 2 to $3\frac{1}{2}$ Kg were studied to determine the effects of metabolic acidemia on digitalis tolerance. The experimental preparation of the cats was identical to that of the puppies. Table IV tabulates the weight, pH, blood gases, and serum potassium in these preparations.

As with the puppies, digitalis tolerance was determined by infusing 40 ug AS/Kg every five minutes thereafter until four consecutive

premature ventricular complexes were observed on the electrocardiogram. The total amount of AS/Kg required to produce toxicity is the digitalis tolerance and these values are tabulated in Table IV for each cat.

The cats, similar to adult animals studied by other investigators (1,2,18) maintained stable body temperature, blood pressure, and electrocardiogram for much longer periods than did the puppies. For this reason, it was possible to use the same cat for two or more consecutive determinations of digitalis tolerance. A two hour waiting period was placed between tolerance tests (32). Each cat could thus serve, for example, as a control and later as an acidemic preparation. Table IV indicates the order of the experiments performed in each cat. There were a total of four control tolerance tests and six metabolic acidosis preparations.

Statistical Evaluation

Digitalis tolerance was computed as µg AS/Kg. Mean control and experimental tolerance values were determined for each litter. Where appropriate, these means were compared with the Student's t test to determine significant differences between control and experimental populations. P values are listed in the various tables under Results.

TABLE I

IDENTIFYING DATA FOR EACH PUPPY

Litter	Puppy	Age(days)	Sex	Wt(kg)	Condition*	Digitalis Tolerance µg AS/KG
I	1	17	M	1.02	C	118
	2	18	F	0.73	C	144
	3	18	F	1.07	C	149
	4	16	M	1.00	RA	180
	5	17	F	1.02	RA	176
	6	19	M	0.68	RA + H	220
II	7	29	F	1.14	C	110
	8	34	F	1.30	C	231
	9	30	F	1.21	RA	351
	10	33	M	1.60	RA	375
III	11	~56	F	2.40	C	83
	12	~56	F	2.50	RA	240
	13	~56	M	3.23	RA	93
IV	14	37	F	1.70	C	88
	15	34	F	0.90	RA	178
V	16	21	M	0.73	C	164
	17	17	M	0.57	C	220
	18	23	M	0.87	C	99
	19	18	F	0.60	RA + H	125
	20	21	M	0.73	RA + H	145
	21	23	M	0.64	RA + H	90
VI	22	32	F	1.70	C	120
	23	33	F	1.42	C	243
	24	28	M	1.58	RA + H	85
VII	25	24	M	1.05	C	352
	26	26	M	1.05	C	343
	27	27	M	1.25	MA	200
	28	32	F	1.50	MA	140
VIII	29	~56	F	2.66	C	170
	30	~56	F	2.54	C	236
	31	~56	F	2.93	C	215
	32	~56	M	3.00	H	190
	33	~56	M	3.68	H	203

* C = control; RA = respiratory acidemia; MA = metabolic acidemia; H = hypoxemia; RA + H = respiratory acidemia plus hypoxemia.

TABLE II(a)

BLOOD GAS, pH, AND ELECTROLYTE VALUES

Litter	Dog	Cond.	Baseline						Acidemia – Hypoxemia						Digitalis Toxicity					
			pH	K	Na	PCO_2	PO_2	O_2Sat.	pH	K	Na	PCO_2	PO_2	O_2Sat.	pH	K	Na	PCO_2	PO_2	O_2Sat.
I	1	C	7.45	3.29	141	31	88	97	–	–	–	–	–	–	7.40	4.78	139	30	91	97
	2	C	7.41	5.79	–	29	97	97	–	–	–	–	–	–	7.39	7.20	–	28	90	96
	3	C	7.31	3.85	140	33	61	88	–	–	–	–	–	–	7.44	6.53	140	25	79	95
	4	RA	7.49	4.48	142	31	70	94	7.19	3.73	145	60	102	93	7.13	6.53	143	67	94	91
	5	RA	7.42	–	–	35	74	95	7.21	4.30	–	66	82	90	7.16	4.10	–	62	79	87
	6	RA+H	7.49	4.06	135	18	92	97	7.19	5.10	131	44	55	70	7.14	5.40	132	46	45	56
II	7	C	7.46	3.41	142	34	61	92	–	–	–	–	–	–	7.41	–	–	27	69	93
	8	C	7.55	3.85	–	27	82	97	–	–	–	–	–	–	7.49	7.42	(113)	25	77	94
	9	RA	7.56	3.84	138	21	90	98	7.15	4.18	135	63	102	93	7.03	7.69	131	77	92	86
	10	RA	7.45	3.76	138	32	69	94	7.25	3.79	137	52	94	94	7.14	7.68	138	54	93	90
III	11	C	7.35	4.80	145	38	74	94	–	–	–	–	–	–	7.41	–	144	31	78	95
	12	RA	7.36	4.28	143	40	72	93	7.13	4.50	142	73	86	88	7.08	5.91	144	80	71	86
	13	RA	7.36	3.76	148	47	82	95	7.13	4.33	146	76	–	–	–	4.98	143	–	63	88
IV	14	C	7.40	3.51	137	41	72	94	–	–	–	–	–	–	7.41	4.46	139	39	82	96
	15	RA	7.46	3.35	139	31	89	97	7.13	3.21	145	73	–	–	7.04	5.64	143	100	79	81

TABLE II(b)

BLOOD GAS, pH, AND ELECTROLYTE VALUES

			Baseline						Acidemia–Hypoxemia						Digitalis Toxicity					
Litter	Dog	Cond.	pH	K	Na	PCO_2	PO_2	O_2Sat.	pH	K	Na	PCO_2	PO_2	O_2Sat.	pH	K	Na	PCO_2	PO_2	O_2Sat.
V	16	C	7.36	4.21	133	41	63	90	–	–	–	–	–	–	–	5.40	128	–	70	88
	17	C	7.40	2.88	133	28	91	96	–	–	–	–	–	–	7.44	5.05	138	29	82	96
	18	C	7.43	3.16	138	31	76	95	–	–	–	–	–	–	7.45	4.36	136	30	72	95
	19	RA+H	7.61	3.00	140	23	116	99	7.07	–	–	80	26	20	7.00	7.52	138	81	31	28
	20	RA+H	7.42	3.33	–	–	72	94	7.12	3.64	–	70	40	50	7.10	6.70	–	68	38	43
	21	RA+H	7.45	2.94	134	24	84	96	7.19	3.57	138	67	44	60	7.12	4.72	138	73	34	37
VI	22	C	7.35	4.2	–	37	66	91	–	–	–	–	–	–	7.36	–	–	32	60	89
	23	C	7.41	3.1	–	34	85	96	–	–	–	–	–	–	7.41	6.9	–	29	78	95
	24	RA+H	7.36	5.0	–	–	–	–	7.22	4.6	–	49	41	60	7.11	7.0	–	46	43	55
VII	25	C	7.43	3.80	137	29	90	97	–	–	–	–	–	–	7.41	7.25	135	21	96	97
	26	C	7.45	3.35	133	28	90	97	–	–	–	–	–	–	7.43	6.40	135	26	90	97
	27	MA	7.40	4.03	139	–	74	94	7.14	4.90	145	31	88	93	6.92	8.00	138	28	84	82
	28	MA	7.46	3.94	139	35	54	88	7.07	4.56	139	31	86	86	6.89	8.10	133	22	89	87
VIII	29	C	7.43	4.07	–	32	83	96	–	–	–	–	–	–	7.41	4.40	–	30	90	96
	30	C	7.40	3.97	–	34	84	96	–	–	–	–	–	–	7.42	–	–	31	94	97
	31	C	7.40	4.10	–	30	94	97	–	–	–	–	–	–	7.42	4.71	–	25	100	97
	32	H	7.38	4.04	–	34	86	96	7.43	4.15	–	27	36	70	7.44	4.50	–	26	30	61
	33	H	7.43	4.07	–	41	76	95	7.49	–	–	33	29	60	7.45	–	–	35	20	38

TABLE III

AVERAGE pH, BLOOD GAS, AND ELECTROLYTE VALUES

FOR EACH EXPERIMENTAL GROUP

Condition	Number	pH	PCO_2*	PO_2*	O_2sat.
Controls	17	7.42 (7.39-7.49)	28.9 (21-39)	82.2 (69-100)	94.9% (88-97)
Respiratory Acidemia	7	7.10 (7.03-7.16)	73.3 (54-100)	81.6 (63-94)	87% (81-91)
Hypoxemia	2	7.45 (7.44+7.45)	30.5 (26+35)	25 (30+20)	49.5% (61+38)
Respiratory Acidemia and Hypoxemia	5	7.09 (7.00-7.14)	62.8 (46-81)	38.5 (31-45)	44% (28-56)
Metabolic Acidemia	2	6.91 (6.89+6.92)	25 (22+28)	86.5 (84+89)	84.5% (82+87)

* PCO_2 and PO_2 values in mm. Hg.

Numbers in parentheses refer to range of values.

TABLE IV

RESULTS OF CAT EXPERIMENTS

Cat	Wt.(Kg.)	Condition*	Digitalis Tolerance**	pH	PCO_2	PO_2	O_2sat
1a	2.3	C	65	7.68	11	100	98
b		MA	54	7.14			
2a	2.1	C	76	7.40	10	102	97
b		MA	38	7.00	22	109	97
c		(C)	57	7.30	27	102	96
d		MA	38	7.16	23	106	96
3a	2.05	C	107	7.38	23	115	98
b		MA	38	7.12	25	126	98
4a	3.49	MA	48	7.20	34	89	94
b		MA	58	7.22	39	78	91

C = Control, MA = Metabolic acidemia

* PCO_2 and PO_2 in mm.Hg.

** Digitalis Tolerance in µg AS/Kg.

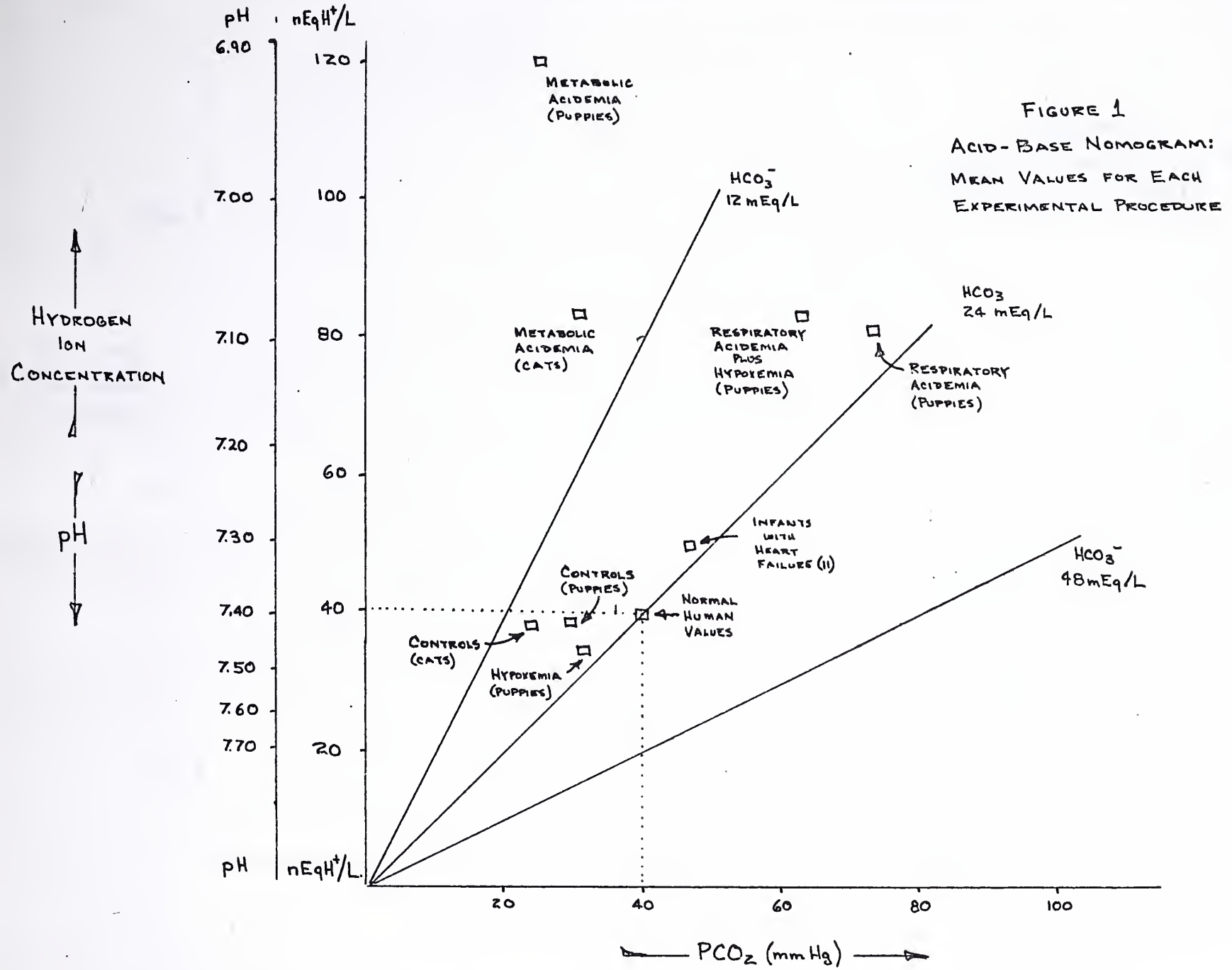

FIGURE 1

ACID-BASE NOMOGRAM: MEAN VALUES FOR EACH EXPERIMENTAL PROCEDURE

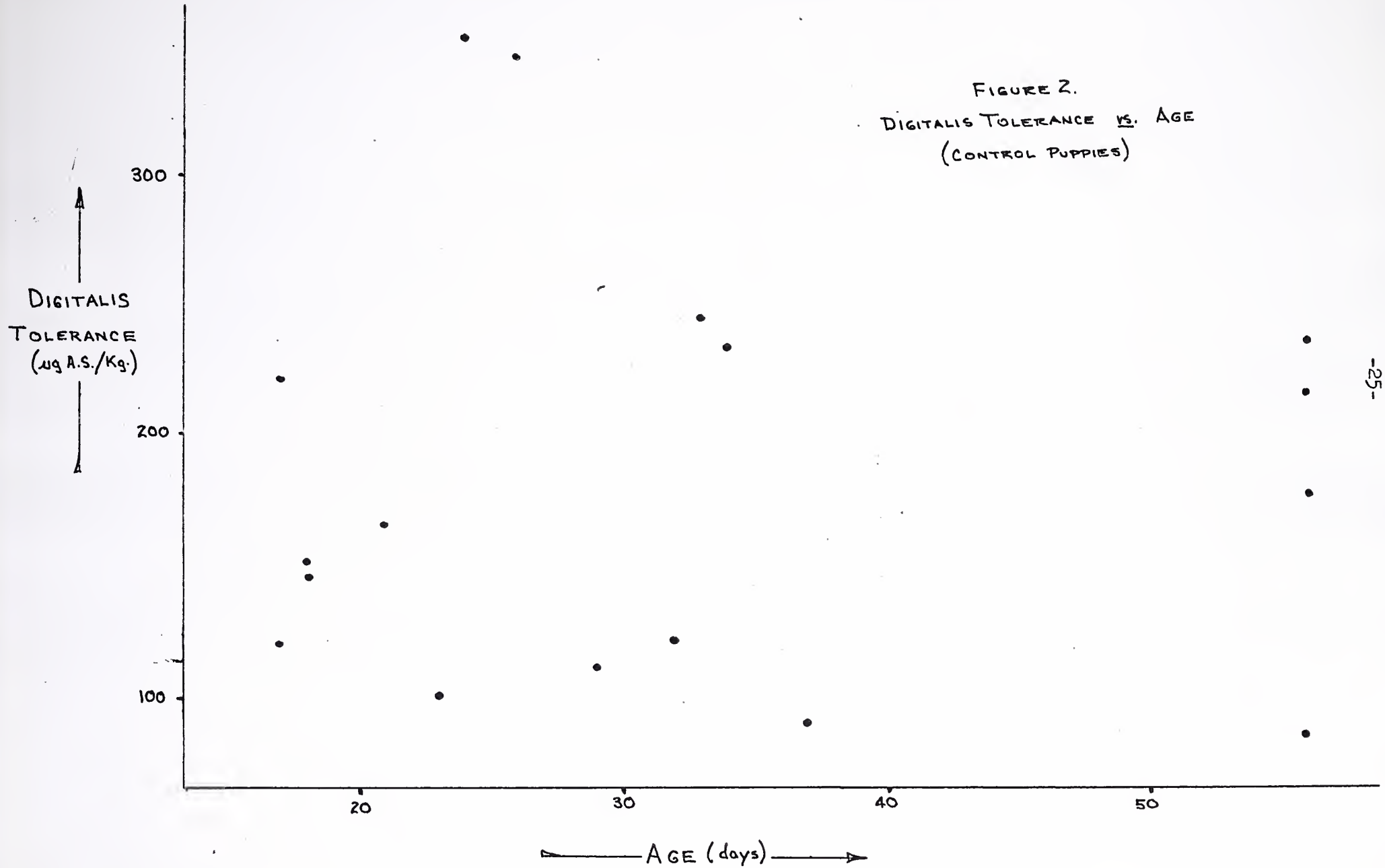

FIGURE 2.
DIGITALIS TOLERANCE vs. AGE
(CONTROL PUPPIES)

RESULTS

A. Comparison of Digitalis Tolerance in Control and Experimental Puppies

The table below groups all puppies from all litters on the basis of their blood gas - pH status, thus all 18 control puppies from all litters are averaged together as one group, all 7 respiratory acidemia puppies are grouped together, etc.

		Digitalis Tolerance (µg AS/Kg)	
Group		Group Average	Range
18 Controls	(C)	181.5	83-352
7 Resp. Acidemia	(RA)	227.6	93-375
2 Hypoxemia	(H)	196.5	190 & 203
5 Resp. Acidemia Hypoxemia	(RA + H)	133.0	85-220
2 Metabolic Acidemia	(MA)	170.0	140 & 200

Inspection of this table suggests that, compared to the control group, respiratory acidemia leads to an increased digitalis tolerance, that respiratory acidemia plus hypoxemia leads to no change or to a decreased digitalis tolerance, and that hypoxemia plus metabolic acidemia lead to no change in digitalis tolerance.

However, an analysis of variance of the control puppies indicates that they do not form a homogeneous group with respect to digitalis tolerance; therefore, it is necessary to consider each litter independently, comparing littermate control and experimental puppies.

Note that the digitalis tolerance is expressed as µg AS/Kg to eliminate variation due to weight among the puppies. There were not sufficient numbers of puppies to determine if sex was a significant variable. The age of the puppies has been plotted on Figure 2 showing that there is no apparent correlation between age and digitalis tolerance in these puppies.

NOTE: The following tables and discussion refer to individual litters of puppies.

Respiratory Acidemia

Respiratory acidemia was tested in four litters. The results of the digitalis tolerance test are tabulated below.

	Digitalis Tolerance (µg AS/Kg)	
Litter	Controls	Experimental
I	118 144 149 (Avg = 137)	180 176 (Avg = 178)
II	110 231 (Avg = 170)	351 375 (Avg = 363)
III	83	240 93 (Avg = 157)
IV	88	178

In each litter, digitalis tolerance was higher in the puppies with respiratory acidemia than their littermate controls. Statistical analysis of individual litters shows that the increased tolerance is significant ($p < .05$) in litter I and shows a trend ($p < .10$) in litter II. The other litters are too small for individual litter analysis but an overall analysis (kindly performed by Irving Miller, Ph.D.,

Department of Biostatistics, Yale University) of the four litters with appropriate weighting to balance for the unequal numbers of puppies in each litter, indicates that respiratory acidemia significantly increased tolerance to acetyl strophanthidin ($p < .02$).

2. Hypoxemia

Hypoxemia was tested in one litter.

	Digitalis Tolerance (µg AS/Kg)	
Litter	Controls	Experimental
VII	215 236 170 (Avg = 207)	203 190 (Avg = 196.5)

Statistical analysis indicates that there is no real difference in tolerance to acetyl strophanthidin between the control and hypoxemic puppies.

3. Respiratory Acidemia Plus Hypoxemia

This was tested in three litters but only litter V had more than one experimental puppy.

	Digitalis Tolerance (µg AS/Kg)	
Litter	Controls	Experimental
V	164 220 99 (Avg = 161)	125 145 90 (Avg = 120)
I	118 144 149 (Avg = 137)	220
VI	120 243 (Avg = 181.5)	85

Only litter V is large enough for statistical analysis; which indicates no significant difference between the control and

experimental puppies. Note that one experimental puppy in litter I has a higher tolerance than its control mean whereas the one experimental puppy in litter VI has a decreased tolerance. Combined statistical analysis (by Dr. Irving Miller) of the three litters fails to reveal a significant variation between control and hypoxemic animals.

4. Metabolic Acidemia

Only one litter was used to test metabolic acidemia.

	Digitalis Tolerance (μg AS/Kg)	
Litter	Controls	Experimental
VII	353 343 (Avg = 348)	200 140 (Avg = 170)

Analysis of this litter indicates that the metabolic acidemia reduced digitalis tolerance ($p < .05$) when compared to the control puppies.

The data from the four experiments show that respiratory acidemia lead to an increased digitalis tolerance; metabolic acidemia in the one litter studied lead to a decreased digitalis tolerance; and hypoxemia and respiratory acidemia plus hypoxemia lead to no statistically significant change in digitalis tolerance. It should be noted that in the last three mentioned experiments, the statistical analysis is based on one litter in each case. The statistics apply to that particular litter only. For example, the statement that metabolic acidemia lead to a decreased tolerance means that, were the litter larger, chances are that the same results would occur in the added

puppies. It does not mean, however, that these results should be necessarily expected to occur in other litters. That assumption must be based on observations of more than one litter. In the four litters with respiratory acidemia, all litters showed the same trend toward increased tolerance and combined analysis showed an overall tendency toward increased tolerance. Thus, it is reasonable to generalize that in other litters of puppies treated equivalently, respiratory acidemia would have the same effect.

B. Potassium in Puppies

All of the sodium and potassium values determined for each dog are tabulated in Table 2. Below are averaged potassium values from the larger litters.

		Serum Potassium (mEq/L)		
Category and Litter		Baseline	Acidemia or Hypoxemic	Toxicity
Respiratory Acidemia (Litter I)	Control	4.31	---	5.84
	Experimental	4.48	4.01	5.34
Hypoxemia (Litter VIII)	Control	4.05	---	4.71
	Experimental	4.06	?	?
Resp. Acidemia and Hypoxemia (Litter V)	Control	3.42	---	4.94
	Experimental	3.09	3.61	6.31
Metabolic Acidemia (Litter VII)	Control	3.58	---	6.82
	Experimental	3.66	4.73	8.05

1. Effect of acidemia on hypoxemia on serum potassium, i.e., comparison of baseline to acidemic values.

a. Respiratory acidemia	4.48 to 4.01 or a fall of 0.47
b. Hypoxemia	? ? ?
c. Respiratory acidemia and hypoxemia	3.09 to 3.61 or a rise of 0.52
d. Metabolic acidemia	3.66 to 4.73 or a rise of 1.07

From these data it appears that only in the case of metabolic acidemia was there a substantial rise in serum potassium.

2. Effect of digitalis on serum potassium in control puppies, i.e., comparison of baseline to toxicity values.

a. Respiratory acidemia	4.31 to 5.84 or a rise of 1.53
b. Hypoxemia	4.05 to 4.71 or a rise of 0.66
c. Respiratory acidemia and hypoxemia	3.42 to 4.94 or a rise of 1.52
d. Metabolic acidemia	3.58 to 6.82 or a rise of 3.24

In each case, acetyl strophanthidin given to "tolerance" tended to produce a rise in serum potassium.

3. Comparison of control and experimental puppies serum potassium at time of toxicity.

a. Respiratory acidemia	5.84 vs. 5.34 or a difference of 0.50
b. Hypoxemia	? ? ?
c. Resp. acidemia and hypoxemia	4.94 vs. 6.31 or a difference of 1.37
d. Metabolic acidemia	6.82 vs. 8.05 or a difference of 1.23

The serum potassium was not significantly different between the control puppies and the respiratory acidemia puppies. There was a higher serum potassium in the experimental puppies, compared to their littermate controls, in the hypoxia, respiratory acidemia plus hypoxemia, and the metabolic acidemia litters.

4. Comparison of potassium rise from baseline to toxicity in control puppies versus acidemic to toxicity in experimental puppies.

	Controls	vs.	Experimentals
a. Respiratory acidemia	1.53		1.33
b. Hypoxemia	---		---
c. Resp. acidemia plus hypoxemia	1.52		2.70
d. Metabolic acidemia	3.24		3.32

When examined in this fashion, the rise in serum potassium, secondary to digitalis infusion to toxicity is about the same regardless of whether the puppy is a control or acidemic animal.

NOTE: It has been observed by the Clinical Chemistry laboratory, where electrolyte determinations were performed, that dog blood has a strong tendency to hemolyze. There may have been some error introduced into the potassium determinations by minimal hemolysis (there are no entries in the tables when gross hemolysis occured); gross trends, however, are probably valid.

C. Results of Digitalis Tolerance in Cats

	Digitalis Tolerance (µg AS/Kg)	
Cat	Control	Metabolic Acidemia
1	65	54
2	76	38
	57	38
3	107	38
4	---	48
		58

Analysis of the data above shows that metabolic acidemia leads to a decreased digitalis tolerance ($p < .05$) compared to the control state. These results in the cat are comparable to the results with metabolism acidemia in the puppy and tend to suggest a general trend that metabolic acidemia with stable PCO_2 leads to a decreased digitalis tolerance.

DISCUSSION

The results of these experiments raise some interesting questions: Why did a decreased pH secondary to hypercapnia raise the digitalis tolerance yet infusion of hydrochloric acid lower the tolerance? Why, since digitalis tolerance rose with "pure" respiratory acidemia, did it not rise with the combination of hypercapnia plus hypoxemia? This question implies that the addition of hypoxemia to hypercapnia does affect the digitalis tolerance, but why did hypoxemia alone have no effect on digitalis tolerance? What possible role, if any, did the serum potassium changes have on digitalis tolerance; and, if potassium is the determining factor in altering tolerance as has been suggested by others, why did tolerance rise in respiratory acidemia and fall in metabolic acidemia when the serum potassium rose in both conditions?

The work of Areskog (36) suggested that carbon dioxide levels modify the inotropic effect of digitalis and the clinical observations cited earlier (4,5,6,41) suggested that respiratory acidemia lead to decreased tolerance and decreased therapeutic effects. Karis, et al. (42) and Hecht and Hutter (43) have indicated the importance of pH manipulations secondary to PCO_2 or bicarbonate alterations in the digitalis augmented automaticity of Purkinje fibers. The puppy experiments also indicate that carbon dioxide is important in determining digitalis activity. These experiments should not be considered as representing a patient with respiratory acidosis, however, because such a patient probably has numerous others acute and chronic medical problems not reproduced here.

Metabolic acidemia without significant alterations of carbon dioxide tension lowered the digitalis tolerance in both the puppies and cats. This appears to be variance with the work of Shaefer, et al. (1) who infused ammonium chloride into adult dogs and observed an increased tolerance to acetyl strophanthidin. They reduced the pH to about 7.3 and did not attempt to maintain a constant PCO_2, but it can probably be assumed that the dogs hyperventilated in response to the acid infusion. Bliss, et al. (2) infused hydrochloric acid to a pH of 7.1 in adult dogs as was done in this experiment with adult cats. (The puppies reached a pH of 6.9). These dogs, however, did not have controlled ventilation. Both groups of investigators found an increase in digitalis tolerance whereas both the cats and puppies in the metabolic acidemia experiments had a decreased tolerance but a controlled PCO_2.

Comparing the puppy experiments; the control pH was 7.4 (40 nEq/L); the respiratory acidemia showed a twofold increase in acidity (pH 7.1, 80 nEq/L); and the metabolic acidemia showed a threefold increase (pH 6.9, 120 nEq/L). One might suggest that a moderate rise in hydrogen ions (eg., respiratory acidemia) lead to an increased tolerance whereas a marked rise in hydrogen ion concentration (eg., metabolic acidemia) overwhelmed the cellular defences and produced a decreased tolerance. However, the metabolic acidemia experiments in the cats refutes this hypothesis because a decreased tolerance still occured with "only" a moderate pH drop (pH 7.1, 80 nEq/L). It seems clear, therefore, that the factors producing and altering the acidemia, eg., PCO_2 and bicarbonate levels, but

not the blood hydrogen ion content per se, are the important factors in determining the digitalis tolerance.

Figure 1, a nomogram (45), is presented to indicate the interaction of carbon dioxide and bicarbonate in altering the pH and the digitalis tolerance.

The work of Adler, Schwartz, Relman, and others (47,48,49) may be of significance. The hydrogen ion, like sodium and potassium, has a transmembrane concentration gradient with the intracellular pH being about 6.8 (about 150 nEq/L of hydrogen ions) with the extracellular pH at 7.4 (40 nEq/L). Maintenance of this hydrogen ion gradient depends, at least, on normal body temperature, aerobic metabolism, and an extracellular buffering system that maintains the blood pH between 7.50 and about 7.10. Within these ranges of blood pH, there are no measurable changes in intracellular pH. An extracellular pH drop below 7.10 secondary to high PCO_2 leads to a drop in intracellular pH; however, intracellular pH does not change in metabolic acidosis until the blood pH drops below 6.9 (47,48,49). In reports noted above (42,43), pH changes have been shown to alter the resting potential and to increase automaticity in Purkinje cell preparations. These reports, however, did not clearly differentiate between respiratory and metabolic types of pH change. It may be that variations in automaticity and membrane potential due to the PCO_2 and HCO_3 concentrations both intra and extracellularly are the significant factors in the altered digitalis tolerance observed with metabolic and respiratory acidemia.

Shafer, et al. (1), and Bliss, et al. (2) suggest that the increased serum potassium observed in their experiments with acidemic dogs produced the increased digitalis tolerance. The experiments reported here are at variance with such a suggestion because serum potassium rose in metabolic acidemia yet tolerance fell. Certainly potassium is important to the action of digitalis and it is well known that infusion of potassium chloride will suppress a digitalis induced arrhythmia. However, increased serum potassium secondary to infusion of potassium is not necessarily equivalent to increased blood levels from the cellular loss of potassium secondary to acidosis, digitalis, or both. It appears that potassium alterations are a response to the acidosis and the digitalis infusion and that the potassium, like the hydrogen ion, is not directly responsible for the observed differences in digitalis tolerance in these experiments.

It is interesting that the experiments with hypoxemia alone did not produce a change in digitalis tolerance. Conn (34) has shown that hypoxia leads to significant alterations in the transmembrane sodium and potassium concentrations and the membrane potential. We might expect, therefore, that hypoxemia might alter the action of digitalis via alterations in membrane potential. Nevertheless, hypoxemia alone had no measurable effect, but when combined with respiratory acidemia there was no increased tolerance as observed with hypercapnia alone. This is evidence that oxygen tension does have some effect and further emphasizes the observation that digitalis tolerance is dependent, at least, on the total interaction of all the blood gas and acid-base parameters.

SUMMARY

Infants in cardiac failure, compared to adults in failure, require higher doses of digitalis (measured in mg./Kg.) to produce digitalis toxicity. The acid-base and oxygen tension alterations observed in some infants with heart failure may be related to the increased digitalis tolerance.

To test this hypothesis, the effect of acute changes of pH, PCO_2, and PO_2 levels on digitalis tolerance were measured in puppies and cats.

Respiratory acidemia without hypoxemia produced an increased digitalis tolerance, whereas metabolic acidemia without hypoxemia produced a decreased digitalis tolerance. Serum potassium, at the time of toxicity, was increased over control values in both respiratory forms of acidemia. Hypoxemia and hypoxemia in combination with respiratory acidemia did not appear to alter digitalis tolerance.

These results suggest that pH, PCO_2, and PO_2 interactions affect the amount of digitalis which will produce toxicity.

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