THE COMPARATIVE DISTRIBUTION OF ISOTOPIC ELECTROLYTES IN GUINEA PIGS DURING HISTAMINE SHOCK

Thesis for the Degree of M. S.

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Marvin Murray

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This is to certify that the

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THE COMPARATIVE DISTRIBUTION OF ISOTOPIC ELECTROLYTES IN GUINEA PIGS DURING HISTAMINE SHOCK

presented by

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has been accepted towards fulfillment of the requirements for

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Wm Dwyne Collings
Major professor

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THE COMPARATIVE DISTRIBUTION OF ISOTOPIC ELECTROLYTES IN GUINEA PIGS DURING HISTAMINE SHOCK

by

MARVIN MURRAY

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INTRODUCTION

Recent progress in chemical and physical methods has given the physiologist a better opportunity to continue investigations of the functions of the organism at the cellular level. Current advances in atomic physics have provided both materials and interest in isotopic tracer investigations. Consequently a tool has been provided for the study of elemental dynamics in the organism.

Since the early days of protein chemistry, it has been known that protein reactions per se depend upon minute quantities of cations and anions, which moderate and direct protein actions and interactions. This has been found to be true in natural reactions as well as those which occur outside the organism.

In the field of immunology, it is known that antigen-antibody reactions depend upon the presence of salts. The exact nature of the relationship between the cations and anions to reactions of proteins has never actually been established, although some investigators have tried to define such a relationship (Szent-Gyorgyi, 1949).

The past half century has shown varied interest in anaphylaxis, histamine and peptone shock and related phenomena. A few authors have attempted to show some relationship between minerals and the above phenomena. Chiefly because of the lack of other

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methods, investigators were confined to studying mineral concentrations in the blood or tissues. For the most part these studies were indecisive.

The principal cations which react with proteins in the organism are sodium, potassium, calcium and magnesium. The ions are contained in and act physiologically in one or more of the organism's phases. It is just as important to establish the mineral dynamics in physiological states as well in pathological conditions such as anaphylaxis and related phenomena, where the barriers which contain the normal phases are altered.

The present work was therefore undertaken to investigate the dynamics of calcium redistribution in the various physiological fluid compartments during a phenomenon such as histamine shock. The major premise at the outset was that such an anaphylactoid reaction, which is probably accompanied by a distinct increase in membrane permeability, should show an ionic redistribution of some sort. Iodine space was determined as a gross experimental control. This ion was chosen because of its availability, and wealth of previous investigation of its distribution in the various body compartments. It is the consensus of opinion that the bulk of iodine in the organism exists in the extracellular fluid with the exception of the intracellular thyroid pool. Thus, a comparison of calcium and iodine distributions should be worthwhile.

Perhaps the first difficulty the investigator en-

counters in reviewing pertinent literature is in the division of topics. Can one group anaphylaxis, histamine shock, peptone shock, trypsin shock, allergy, hypersensitivity, and other like reactions in one general category, or should one specifically separate them and treat them individually? Apparently it seems to be a matter of convenience, and there is a general tolerance of each viewpoint. In view of the general scarcity of literature available with reference to this specific topic and lack of proof that these reactions are not similar mechanisms, it would seem expedient to discuss the group as highly similar phenomena.

The very nature of the relationship between calcium and the aforementioned phenomena has been a highly debatable subject. Various investigators align themselves into three groups: those who claim an increase of blood calcium during the crisis of the phenomena, those who hold the opposite, and those who say there is no change at all.

Clinically, Brown and Hunter (1925) showed that in a plurality of cases of asthma, hayfever, etc., a definite calcium deficiency existed. Furthermore, they stated that increased dietary calcium, administration of calcium lactate, and thyroid and parathyroid hormone therapy alleviated severe clinical syndromes. Kallos and Kallos-Deffner (1938) showed that injections of calcium to allergic guinea pigs inhibited the responses of isolated uteri and bronchial muscles to specific antigens. Also, these investigators showed that calcium and atropine prevented asthmatic attacks in humans.

Kastle, Healy and Buckner (1943) demonstrated that excess calcium lactate intraperitoneally did not protect guinea pigs from anaphylaxis, whereas small amounts did. Arloing, Langerson, and Mounier-kuhn (1925) showed that injections of calcium chloride at the time of sensitization in no way influenced anaphylaxis. However, if calcium chloride was injected at the time of the shock dose of antigen, shock was attenuated.

A rarely cited work by Schittenhelm, Erhardt, and Warnat (1928) contains a complete account of calcium and potassium levels in blood and tissues of dogs and rabbits during sensitization and anaphylaxis. These data showed that during shock there was a small but definite decrease in blood and serum calcium. Moreover, they found that calcium levels in liver increased, whereas in lung they decreased during shock.

Kuschnaryew (1930) confirmed the data of Arloing et al. (1926) and in addition showed that anaphylaxis was augmented by the injection of potassium chloride. By chemical analyses, Kuschinsky (1929) found that histamine shock produced a slight rise in serum and plasma calcium, however total blood calcium was lowered.

Conflicting with the previous reports, Azzo Azzi (1922) stated that results of his investigations of calcium blood levels in guinea pigs in anaphylactic shock were so varied that no conclusions were possible. Also with guinea pigs, Brown and Ramsdell (1929) reported that total blood calcium did not change during

anaphylaxis, but diffusible calcium increased during shock. Similarly, Averianow (1926) found no deviations in blood calcium of dogs during anaphylaxis.

Still more puzzling are the following reports which indicate that blood calcium increases during anaphylaxis. Anan Sinji (1927) reported raised blood levels in guinea pigs and rabbits during anaphylaxis, histamine and peptone shock. Similarly Drilhon, Cloque, Galup, Debedour (1934) reported that in asthma in humans there was a general increase in blood calcium. In 1939 Yosito Sidara, taking issue with Anan Sinji, reported decreased serum calcium in rabbits during peptone shock. However, he confirmed the previous observations that histamine shock increased blood calcium levels.

METHODS AND MATERIALS

All work was done with male and female guinea pigs varying in weight between 550 and 1100 grams. Four groups of animals were established. Groups I and II were designated "Iodine - 131 control" and "Iodine - 131 histamine" respectively. Groups III and IV were designated "Calcium - 45 control" and "Calcium - 45 histamine" respectively.

The iodine 131 isotope was used in the form of potassium iodine 131 with potassium iodide carrier. The concentration was made by adding the isotopic potassium iodide to physiological saline to form a dilution per 25 ml. which would read 14,750 counts per minute at zero distance from a thin end window Geiger-Muller tube with aluminium filter. The dosage was then defined as 0.5 ml. per one hundred grams of body weight.

The calcium 45 isotope was in the form of calcium 45 carbonate with calcium carbonate carrier. The calcium 45 carbonate was treated with 4 normal hydrochloric acid in distilled water. The pH was adjusted to 6 with the addition of 6 normal sodium hydroxide, and the solution was diluted to 100 ml. The dosage was then established as 0.5 ml. per 100 grams of body weight.

Histamine acid phosphate was used in concentration of 2.75 mg. per ml. The dosage to produce shock was 1 ml. per 1000 grams of body weight.

Iodine Control Group I.

The animals were injected subcutaneously at zero time

with the iodine 131 solution. At two, four, and six hours respectively, 1 ml. of blood was withdrawn by means of heart puncture. The blood was placed in previously tared porcelain crucibles which had been weighed at constant weight. Two sizes of crucibles were used: 00 and 0000. At six hours the animals were sacrificed by asphyxiation with dimethyl ether. The animals were immediately autopsied and samples of liver, lung, bladder, thyroid, uterus, and pectoralis major muscle were taken. These were weighed on a torsion balance accurate to the third decimal.

All of the samples were dried to constant weight in a dry air oven at 102 degrees centigrade. They were placed in a desiccator and weighed on an analytical balance (which had previously been used to weigh all of the crucibles) which was accurate to plus or minus 0.00005 grams. The weights were recorded.

The samples were then measured for radioactivity with a thin end window type Geiger-Muller tube which recorded by means of an electronic register. The samples were shielded in lead counting chambers. Geometry was fixed for small and large crucibles, the air path for the large crucibles was 5 cm. and for the small ones 2 cm. No attempt was made to correct for the obvious discrepancy in geometries, since no direct cross correlations were made between counts. Since all counts were below 1500 per minute, no corrections for coincidental counting were made. The ranges of weights of materials counted indicated that self-absorption was within 2 percent (Lee, 1950).

The samples were then allowed to decay until the

amount of radio-activity was practically nil. Afterward they were ashed in an electric oven at a temperature of 1500 degrees Fahrenheit. The crucibles containing the ash were weighed at constant weight.

Iodine and Histamine Group II.

The procedure followed for this group was exactly the same as in Group I. However, at six hours histamine acid phosphate was injected into the heart to produce shock. Shortly before death, one ml. of blood was withdrawn from each animal by heart puncture.

The animals were autopsied immediately, and the ensuing procedure followed that of Group I.

Calcium Control Group III

The procedure was the same as in Group I with the following exceptions: The animals were injected with isotopic calcium at zero time. All crucibles used in the calcium experiments were of the 0000 size. . After drying the tissues in the dry air oven, they were ashed in the electric muffle furnace at 1500 degrees centigrade. Then, after weighing at constant weight, the samples were measured for radioactivity by the same type Geiger-Muller tube and counter as before.

Calcium Histamine Group IV

The method followed was the same as in Group III, with the exception that death was induced at six hours by means of injection with histamine acid phosphate.

In both calcium experiments, no correction was made for coincidental counting as none of the tissues emanated over

1500 particles per second. The geometry was fixed for all samples and the air path was 2 cm. Self absorption was within 2 percent with reference to curves determined by Atens (1950) and Murray and Refson (1950).

Previous investigators have shown that there is no appreciable variation in calcium content of the erythrocytes during anaphylaxis (Schittenhelm, 1928). However, due to the nature of the experiment it was thought desirable to check the calcium distribution in the red blood cells. Three guinea pigs weighing 1280, 1140, and 1080 grams respectively were injected with calcium 45 chloride subcutaneously at zero time. At six hours, the animals were injected with histamine acid phosphate by heart puncture. During the ensuing spasms, seven ml. of blood were removed from the heart of each animal. The blood was heparinamized and centrifuged. The hematocrit was determined. The supernatant plasma was decanted, and the cells were washed in saline four times. The cells were then dried and ashed and subsequently counted for particle emanation.

Average hematocrits were determined for all four experimental groups.

Standards were prepared of each injection solution of isotopic substance by micropipetting 0.01 and 0.02 ml. in each size crucible and assigning standards for each experimental group. These were then dried or ashed depending on the procedure in the

experimental group, and subsequently counted in the same manner as the samples of tissue.

CALCULATIONS

RADIO DECAY

Determination of the Specific Activity Constant

$$N = N_o e^{-\lambda t_{\frac{1}{2}}}$$

$$\ln \frac{N}{N_o} = -\lambda t_{\frac{1}{2}}$$

$$-\ln \frac{2}{t_{\frac{1}{2}}} = -\lambda$$

N Number of particles emanating after 1 half life

N_o Number of particles emanating at zero time

$t_{\frac{1}{2}}$ Time of half life

λ Specific activity constant

Determination of Decay

$$A = A_o e^{-\lambda t}$$

A Activity at time of measurement

A_o Activity at zero time

t Time

λ Specific activity constant

DETERMINATION OF EXTRACELLULAR SPACE

$$\frac{\text{Activity of 1 ml. standard} \times \text{injection fluid volume}}{\text{activity of 1 ml. of plasma}} = \text{diluting volume (ml.)}$$

Equilibrium of the absorbed materials was arbitrarily assumed to have taken place by two hours following injection, and

the value calculated was equated to unity of extracellular volume. Succeeding volumes were then plotted as percentages of unity.

DETERMINATION OF MINERAL SPACE IN TISSUE
(Mannery and Haege, 1941)

$$\frac{\text{Activity of 1 gm. tissue}}{\text{Activity of 1 ml. plasma}} \times .95 \times .93 \times 100 = H_2O_e^zX \text{ gm./100 gm. fresh tissue}$$

$H_2O_e^zX$ is expressed as the water mineral equilibrium in gm. per hundred grams fresh tissue. 0.95 is the Gibbs-Donnan ratio and 0.93 is the correction for the water content of plasma.

PLASMA COUNTS

The average hematocrit was found to be 45 percent. Therefore, counts per ml. of plasma were calculated as follows:

$$\frac{\text{counts per ml. whole blood}}{.55} = \text{counts per ml. plasma}$$

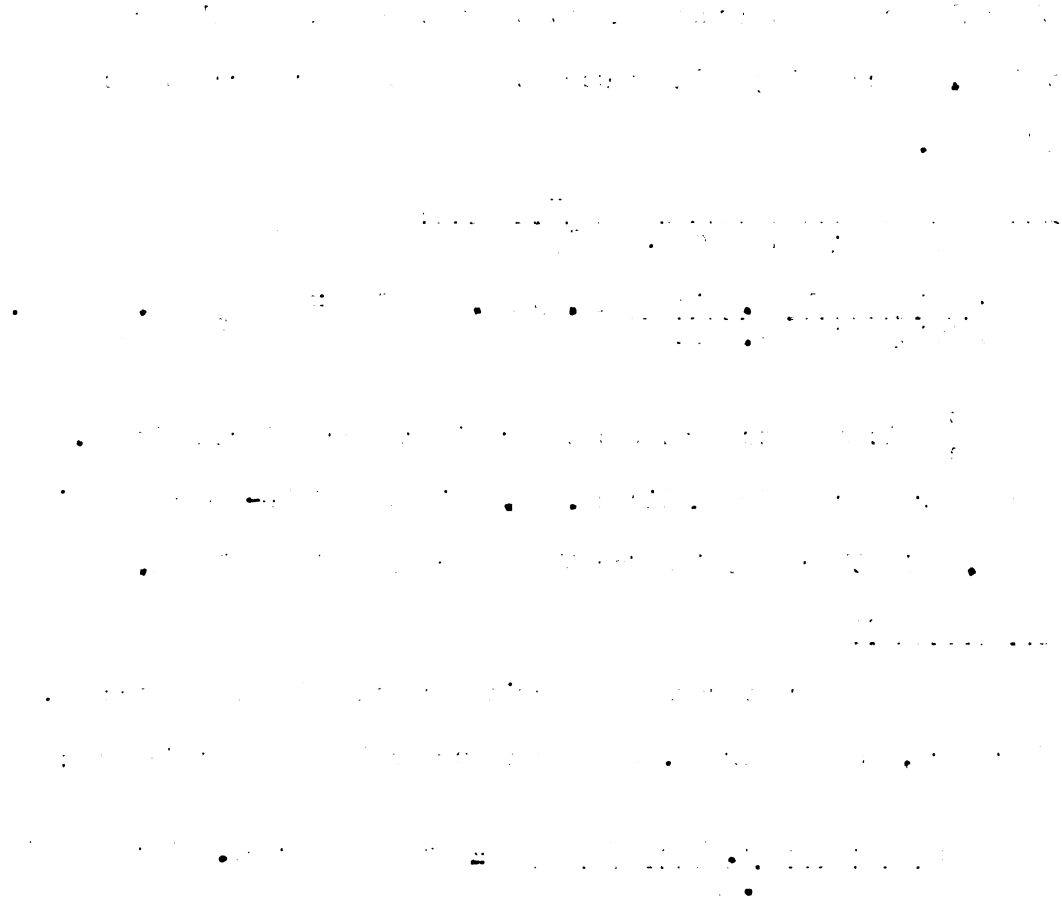


TABLE I THE DISTRIBUTION OF WATER AND ASH IN THE IODINE CONTROL AND IODINE HISTAMINE EXPERIMENTS

Tissue / Animal numbers		Percentage of Water		Percentage of Ash	
Control	Histamine	Control	Histamine	Control	Histamine
Liver					
#1	#3	74.9	71.9	5.6	5.9
#2	#4	76.0	78.0	6.8	5.5
#6	#8	69.5	71.8	7.4	5.2
#8	#10	68.9	72.0	5.1	6.6
X*	X	72.3	73.4	6.2	5.8
Lung					
#1	#3	80.7	77.9	8.3	7.7
#2	#4	78.7	79.2	7.0	12.7
#6	#8	71.4	72.8	10.2	9.5
#7	#10	88.7	77.8	7.2	15.9
X	X	79.9	76.9	8.2	11.5
Bladder					
#1	#3	84.3	82.9	12.4	8.8
#2	#4	84.4	84.6	15.3	10.6
#6	#8	79.0	84.4	12.7	9.7
#7	#10	82.8	80.8	7.6	15.2
X	X	82.6	83.2	12.0	11.1
Pectoral Muscle					
#1	#3	77.4	78.0	8.2	9.1
#2	#4		77.4	8.8	7.5
#6	#8	70.0	76.1	11.0	7.5
#7	#10	70.0	88.3	3.8	10.3
X	X	72.5	80.0	8.0	8.6
Thyroid					
#1	#3	70.5	72.4	5.3	11.6
#2	#4	71.2	72.2	6.9	6.9
#6	#8	67.6	70.7	5.8	8.3
#7	#10	67.1	69.4	8.9	9.7
X	X	69.1	71.2	6.7	9.1
Uterus					
#6			83.7	3.8	14.2
#7		80.5	82.9	6.1	13.5
X			83.3	5.0	13.9

* X = Average

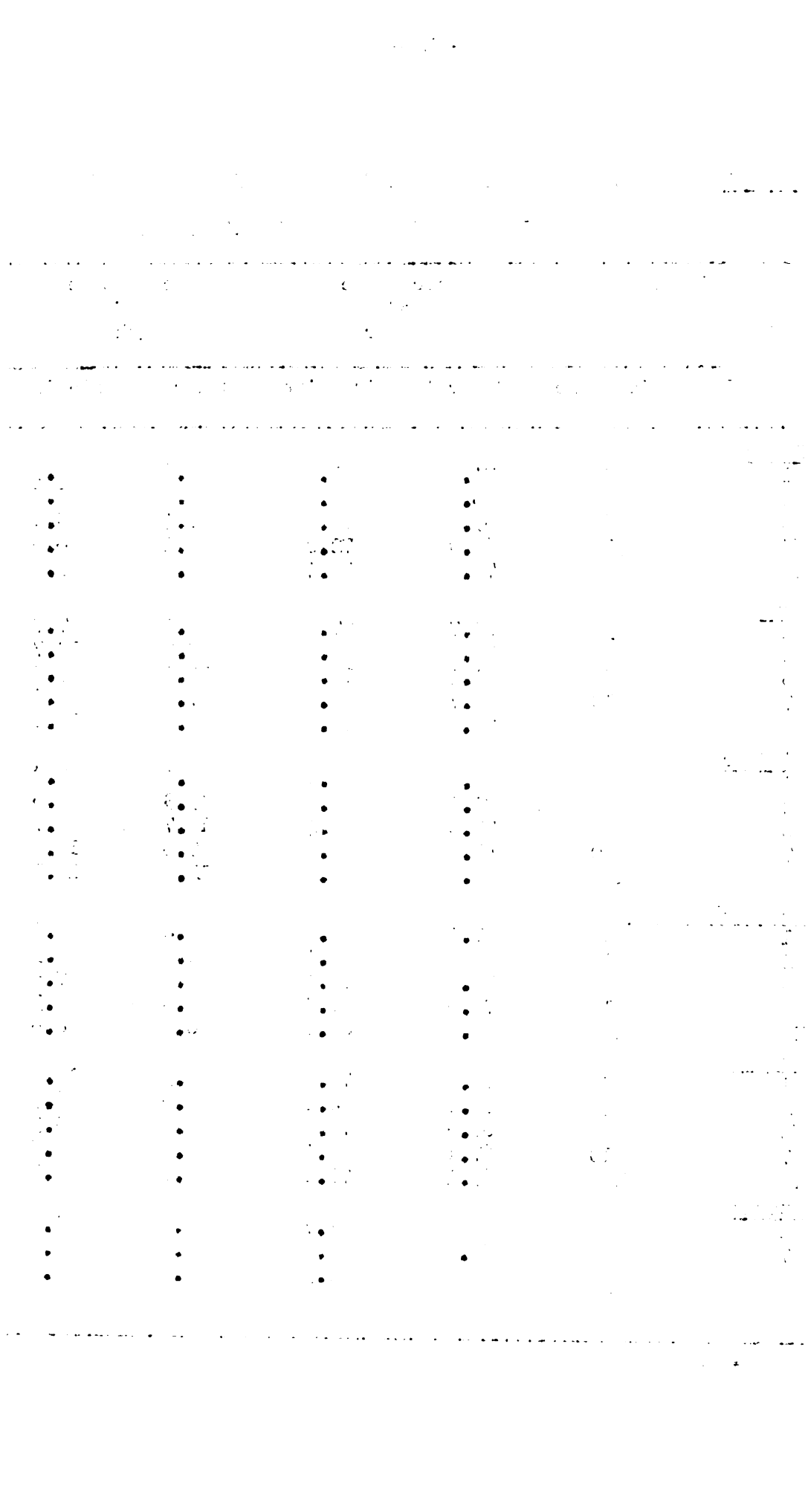


TABLE II COUNTS PER SECOND PER ML. OF WHOLE BLOOD IN IODINE HISTAMINE AND IODINE CONTROL EXPERIMENTS

	*C #1 H #3	C #2 H #4	C #6 H #8	C #7 H #10
2 hr. Control	19.90	7.37	10.69	9.89
2 hr. Histamine	21.23	13.42	12.29	
4 hr. Control	3.96	3.85	11.29	8.75
4 hr. Histamine	7.15	5.50	6.42	10.22
6 hr. Control	2.62	.04	10.25	12.56
6 hr. Histamine	2.42	2.86	8.37	6.85

* C #1 = Control Animal 1, etc.

H #3 = Histamine Shocked Animal Number 3, etc.

TABLE III. THE DISTRIBUTION OF I^{131} IN TISSUE ASH AND IN FRESH TISSUE IODINE CONTROL AND IODINE HISTAMINE EXPERIMENTS

Tissue Animal numbers		Counts/gm. Fresh Tissue Per Second		Counts/mg. Ash Per Second	
Control	Histamine	Control	Histamine	Control	Histamine
Pectoralis Muscle					
C 1	H 3	.30	.00	.02	.00
2	4	.13	.00	.01	.00
6	8	2.04	1.13	.06	.06
7	10	1.64	5.75	.14	.28
Liver					
C 1	H 3	.97	.23	.07	.01
2	4	.96	.63	.06	.05
6	8	3.23	14.10	.14	.96
7	10	.95	3.81	.06	.21
Lung					
C 1	H 3	.98	.99	.06	.06
2	4	1.08	.92	.07	.03
6	8	8.38	7.91	.29	.30
7	10	4.19	11.36	.27	.32
Bladder					
C 1	H 3	1.32	.49	.07	.03
2	4	11.69	.00	.49	.00
6	8	5.56	10.67	.21	.07
7	10	5.28	6.94	.40	.24
Thyroid					
C 1	H 3	23.23	52.41	1.47	1.63
2	4	15.10	37.75	.76	1.97
6	8	264.22	267.85	14.15	10.89
7	10	154.34	199.91	5.28	6.70
Uterus					
C 7	H 10	7.15	6.60	.56	.29

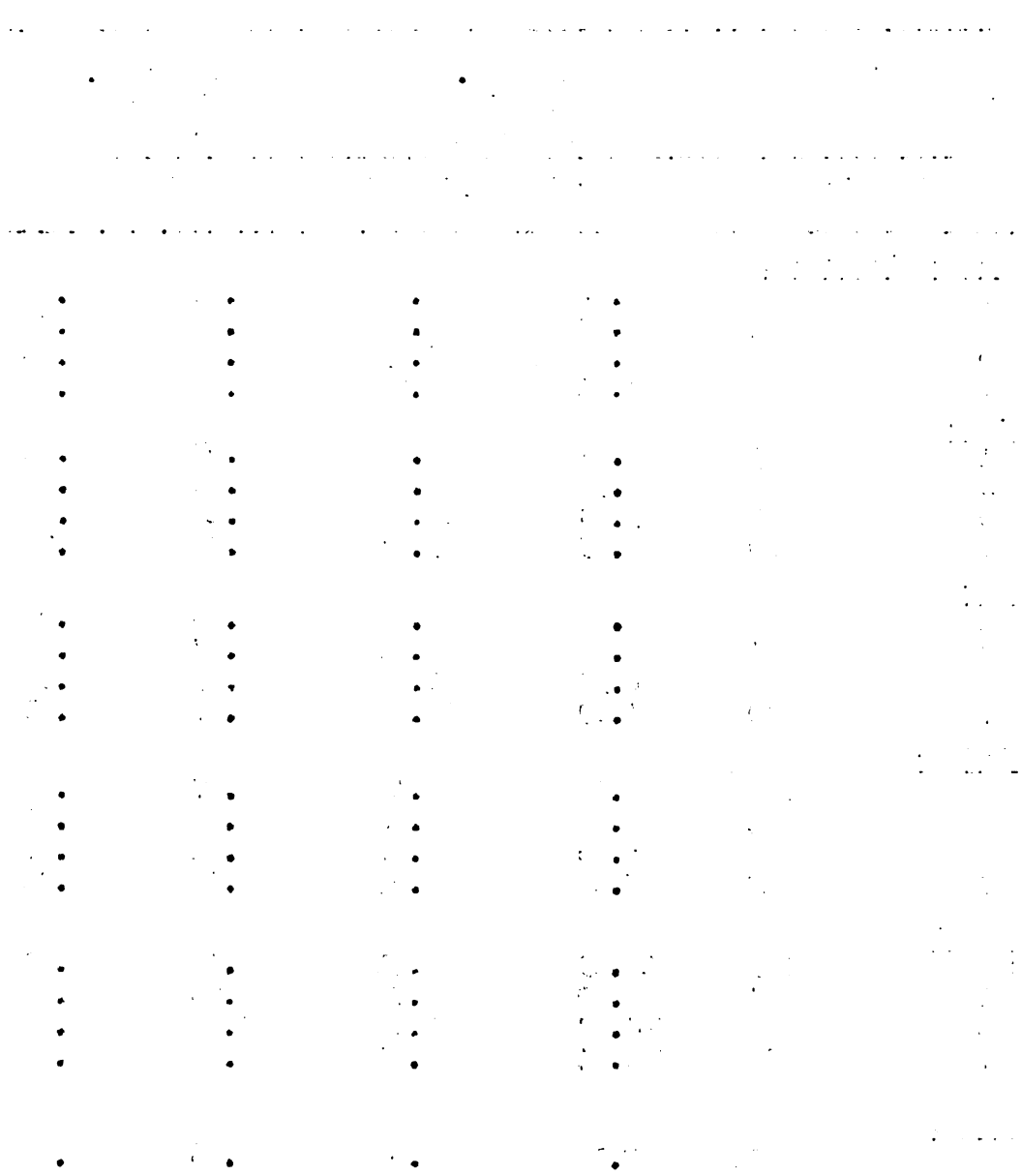


TABLE IV CONTROL VALUES FOR TISSUE SPACE IN IODINE
CONTROL - GROUP I

Animal Number	1	2	6	7	X*
Time	4 hr.	6 hr.	6 hr.	6 hr.	6 hr.
Tissue					
Pectoralis Muscle	1.6	5.7	9.7	9.9	6.7
Liver	11.4	18.1	15.3	5.7	12.6
Lung	13.6	18.0	40.00	25.3	24.2
Bladder	185.0	25.0	27.6	32.0	67.4
Thyroid	190.0	430.0	1250.0	930.0	700.0
Uterus				43.0	

All values given in gm./100 gm. fresh tissue

TABLE IVa EXPERIMENTAL VALUES FOR TISSUE SPACE IN IODINE
HISTAMINE - GROUP II

Animal Number	3	4	8	10	X*
Time	6 hr.	6 hr.	6 hr.	6 hr.	6 hr.
Pectoralis Muscle	0.00	00.0	6.6	4.1	2.7
Liver	4.7	10.7	81.0	27.0	30.9
Lung	20.0	15.7	46.0	81.0	40.7
Bladder	9.9	0.0	60.0	49.5	29.9
Thyroid	292.0	640.0	1525.0	1940.0	1099.0
Uterus			41.0	46.8	43.9

All values given in gm./100 gm. fresh tissue

* X = Average

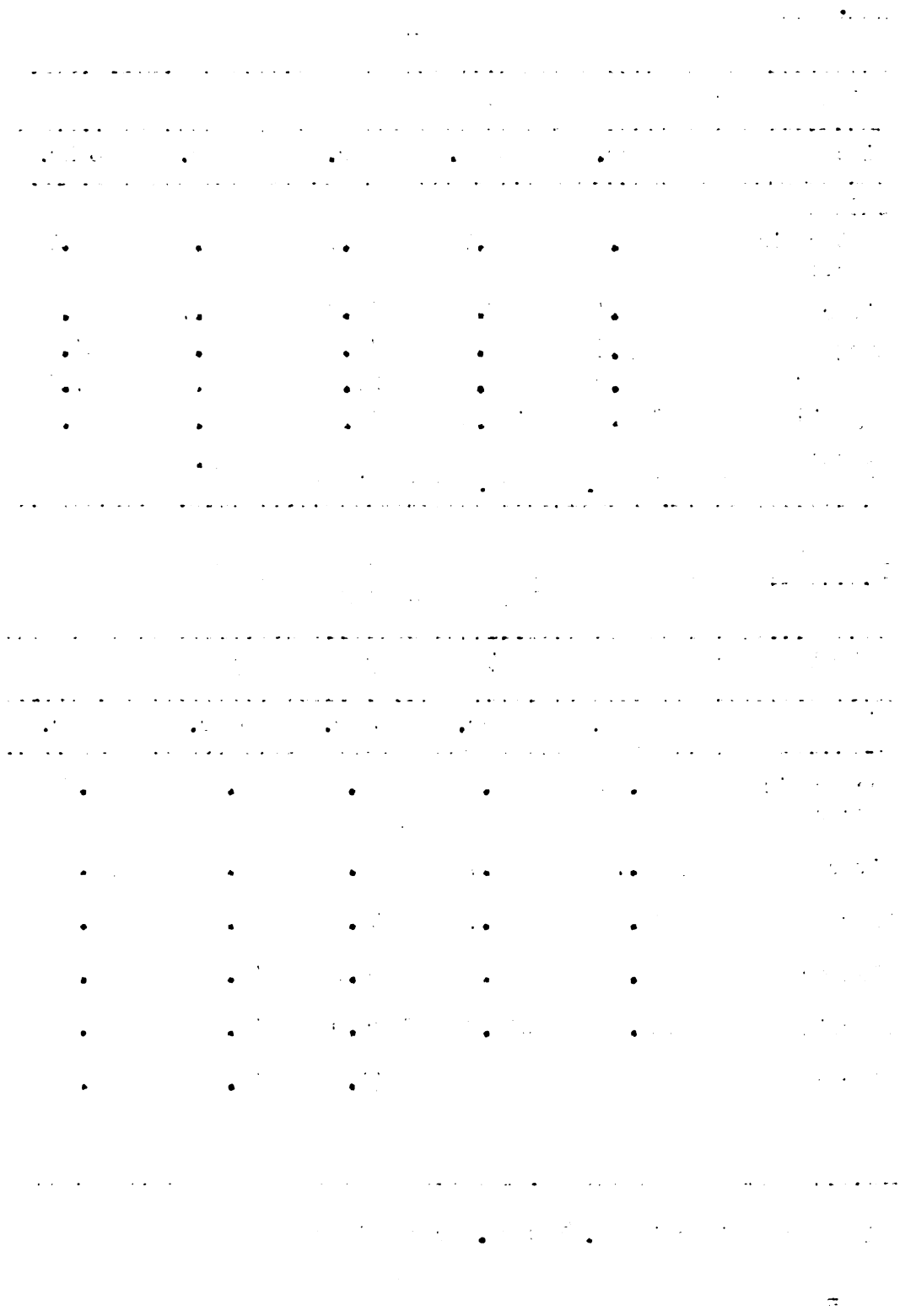


TABLE V. THE DISTRIBUTION OF WATER AND MINERALS IN THE CALCIUM CONTROL AND CALCIUM HISTAMINE EXPERIMENTS

Tissue / Animal Numbers		Percentage of Water		Percentage of Ash	
Control	Histamine	Control	Histamine	Control	Histamine
Pectoralis Muscle					
#1	#3	72.6	77.2	5.8	5.8
#2	#5	73.0	80.5	5.3	5.9
#7	#6	77.6	78.4	9.4	5.5
X *	X	74.4	78.7	6.8	5.7
Liver					
#1	#3	80.1	76.3	7.5	6.3
#2	#5	78.6	27.2	7.3	5.6
#7	#6	69.0	73.7	7.0	5.3
X	X	75.9	75.0	7.3	5.7
Lung					
#1	#3	78.7	80.0	7.3	7.1
#2	#5	79.1	83.0	6.6	7.0
#7	#6	59.8	82.3	6.5	6.9
X	X	72.5	81.8	6.8	7.0
Bladder					
#1	#3	83.3	92.4	5.3	7.2
#2	#5	82.7	86.8	6.3	1.9
#7	#6	82.4	84.7	1.2	8.0
X	X	82.8	88.0	5.8	7.6
Uterus					
#1	#3	83.2		7.6	
#2	#5	81.6		6.2	
#7	#6	79.5	83.0	7.7	6.5
X	X	81.4		7.2	

* X = Average

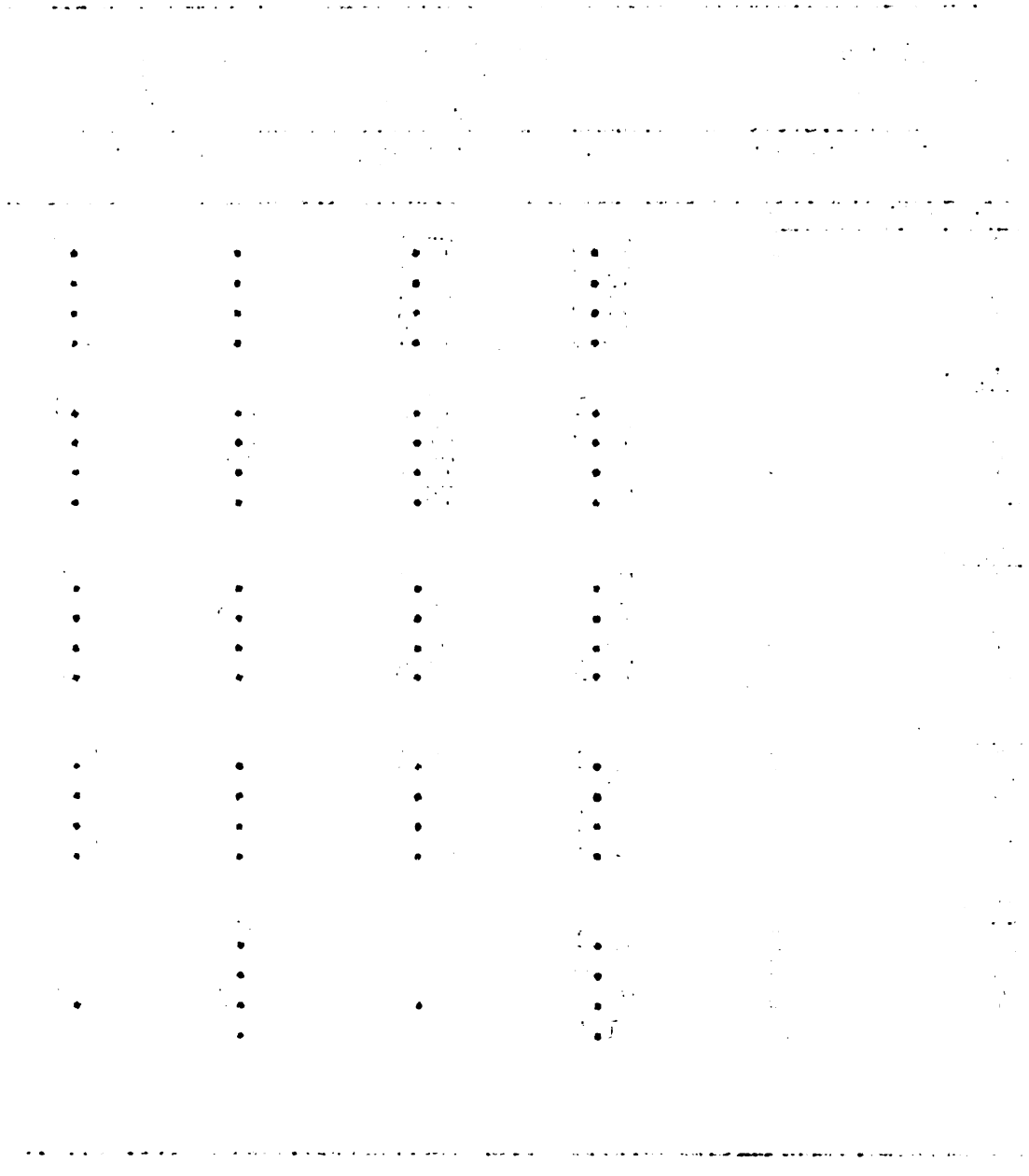


TABLE VI COUNTS PER SECOND PER ML. OF WHOLE BLOOD IN CALCIUM CONTROL AND CALCIUM HISTAMINE EXPERIMENTS

	*C #1 H #3	C #2 H #5	C #7 H #6
2 Hr. Control	10.69	7.05	20.34
2 Hr. Histamine	10.69	7.80	13.61
4 Hr. Control	15.85	6.70	11.41
4 Hr. Histamine	14.85	8.77	15.50
6 Hr. Control	19.52	6.97	
6 Hr. Histamine	11.51	4.15	12.23

*C #1 = Control animal number 1, etc.

H #3 - Histamine shocked animal number 3, etc.

TABLE VII THE DISTRIBUTION OF Ca^{45} IN TISSUE ASH AND FRESH TISSUE CALCIUM CONTROL AND CALCIUM HISTAMINE EXPERIMENTS

Tissue / Animal number		Counts/gm. Fresh Tissue Per Second		Counts/mg. Ash Per Second	
Control	Histamine	Control	Histamine	Control	Histamine
Pectoralis Muscle					
#1	#3	7.47	15.87	.48	1.18
#2	#5	6.14	18.07	.44	1.57
#7	#6		67.47		5.73
Liver					
#1	#3	10.60	11.31	.67	.76
#2	#5	7.28	15.86	.51	.38
#7	#6	30.9	29.84	1.42	2.24
Lung					
#1	#3	50.08	18.30	3.38	1.29
#2	#5	8.79	10.04	.57	.85
#7	#6	54.89	17.11	1.85	1.39
Bladder					
#1	#3	31.79	5.74	3.63	1.05
#2	#5	7.61	12.66	.70	4.92
#7	#6		35.57		2.90
Uterus					
#1	#3	18.25		1.43	
#2	#5	7.56		.66	
#7	#6	41.54	18.63	2.63	1.70

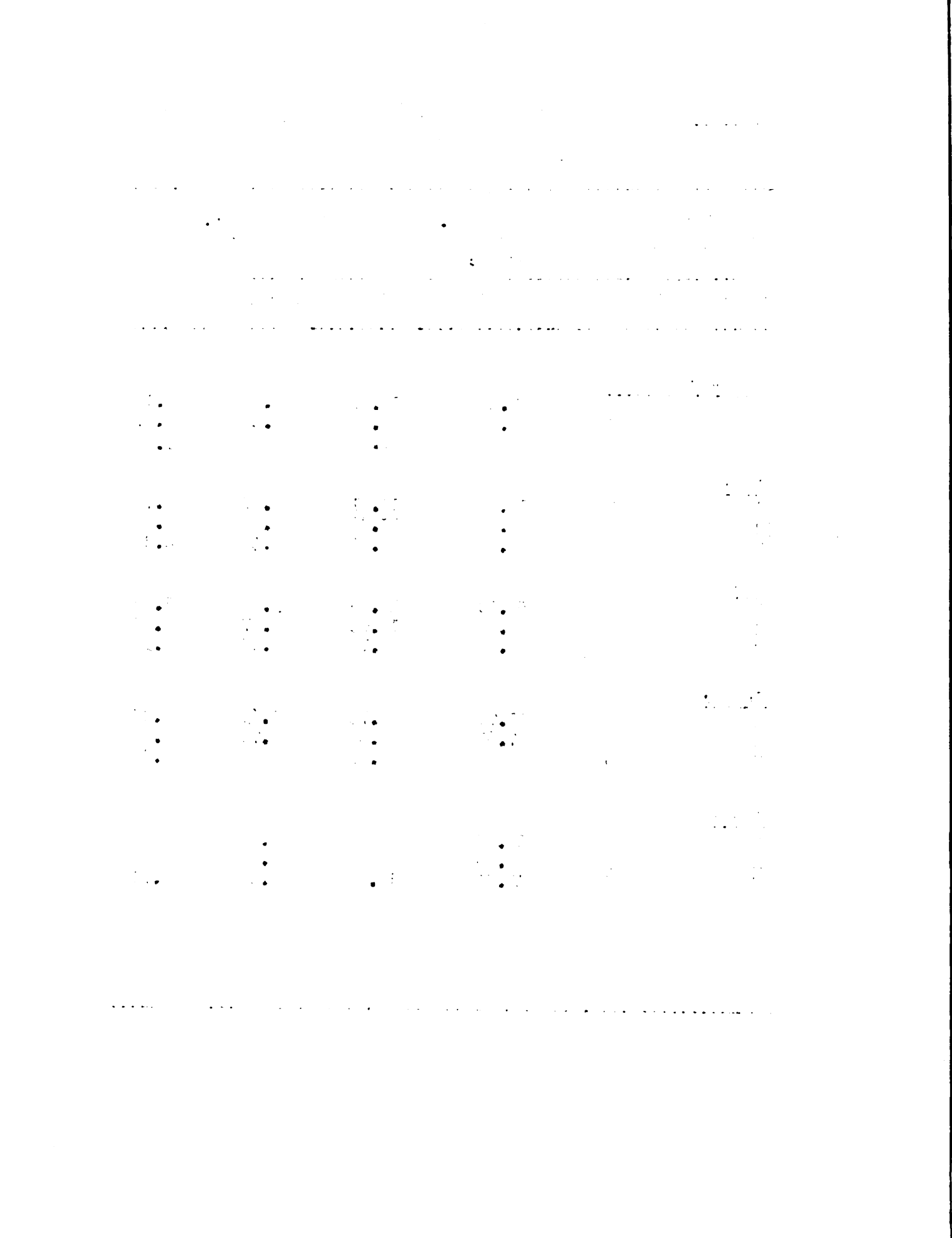


TABLE VIII *CONTROL VALUES FOR TISSUE SPACE IN CALCIUM CONTROL - GROUP III

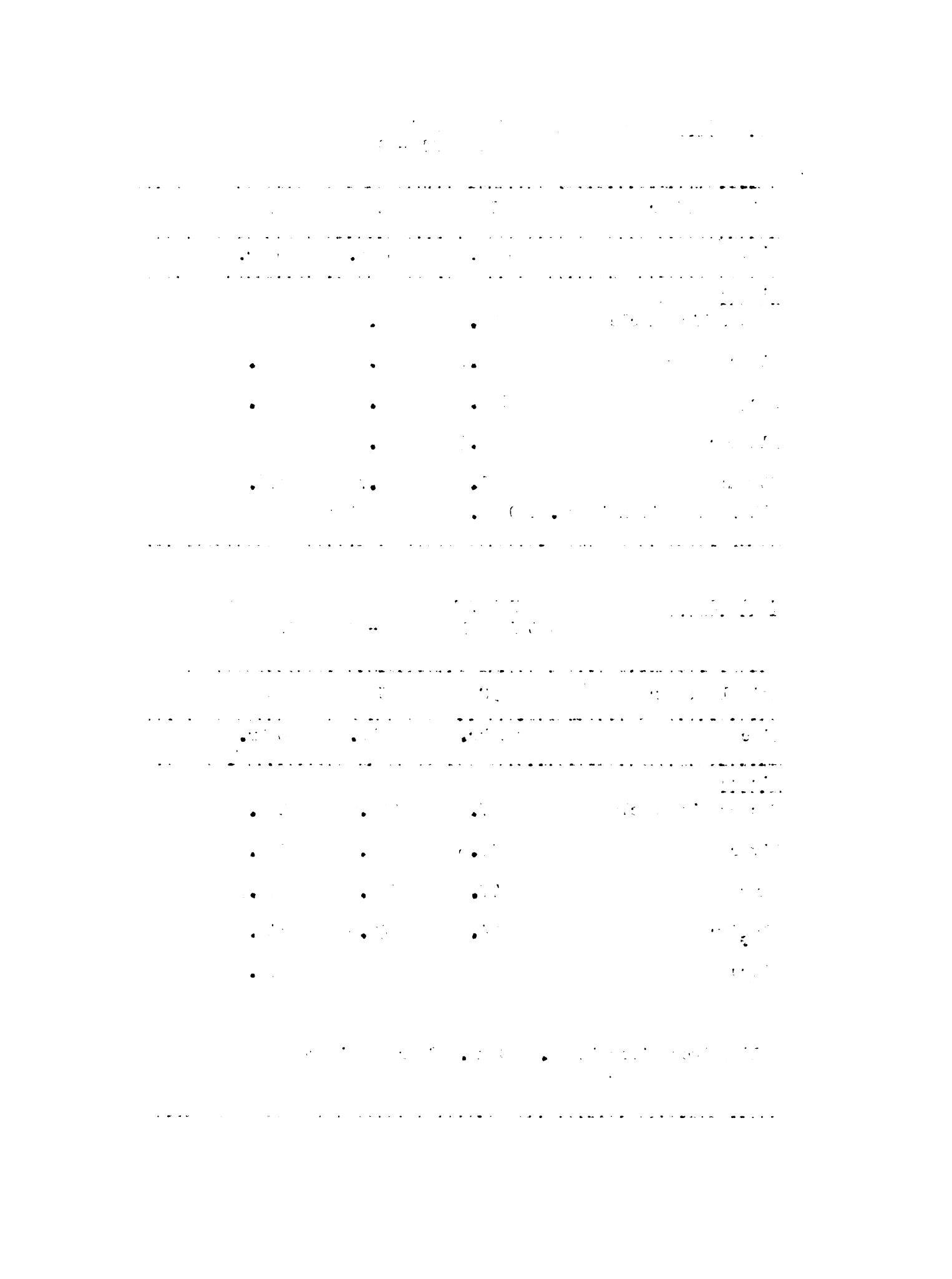
Animal Number	1	2	7
Time	6 hr.	6 hr.	6 hr.
Tissue			
Pectoralis Muscle	20.9	41.8	
Liver	29.7	50.5	130.0
Lung	140.0	61.0	233.0
Bladder	89.4	53.0	
Uterus	51.0	52.7	176.0

*All Values Given in gm./100 gm. of Fresh Tissue

Table VIIIa *EXPERIMENTAL VALUES FOR TISSUE SPACE IN CALCIUM HISTAMINE - GROUP IV

Animal Number	38	5	6
Time	6 hr.	6 hr.	6 hr.
Tissue			
Pectoralis Muscle	67.0	238.0	268.0
Liver	47.6	210.0	109.0
Lung	77.0	106.0	68.3
Bladder	24.2	148.0	142.0
Uterus			74.0

*All Values Given in gm./100 gm. of Fresh Tissue



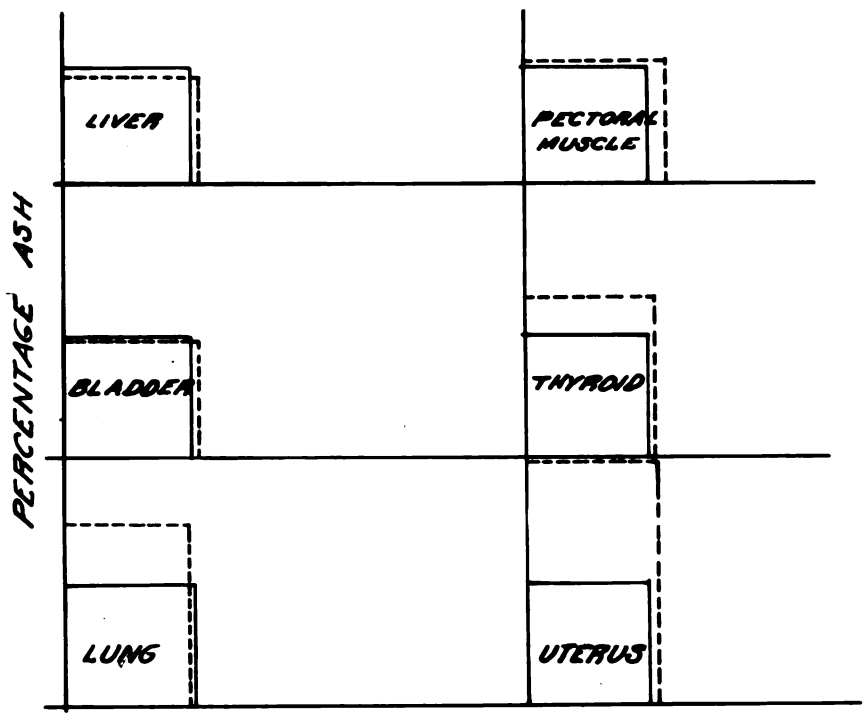


FIG. 1
RELATIVE WATER-MINERAL SHIFTS DURING HISTAMINE SHOCK.

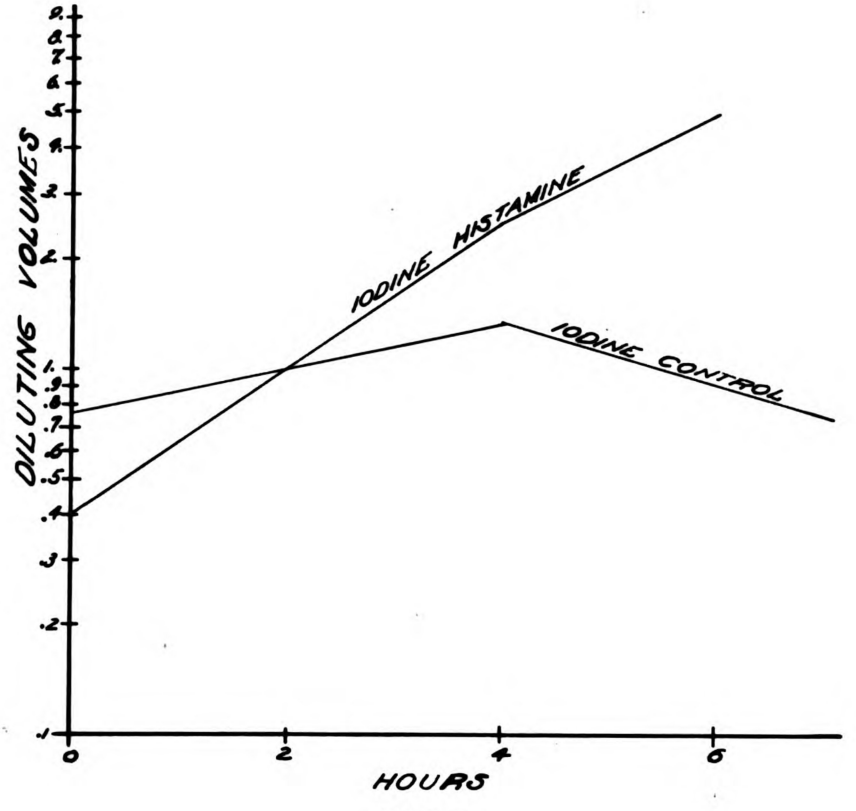


FIG. 2

COMPOSITE CURVES SHOWING EFFECT OF HISTAMINE UPON THE VOLUME OF DILUTING FLUID FOR INJECTED IODINE.

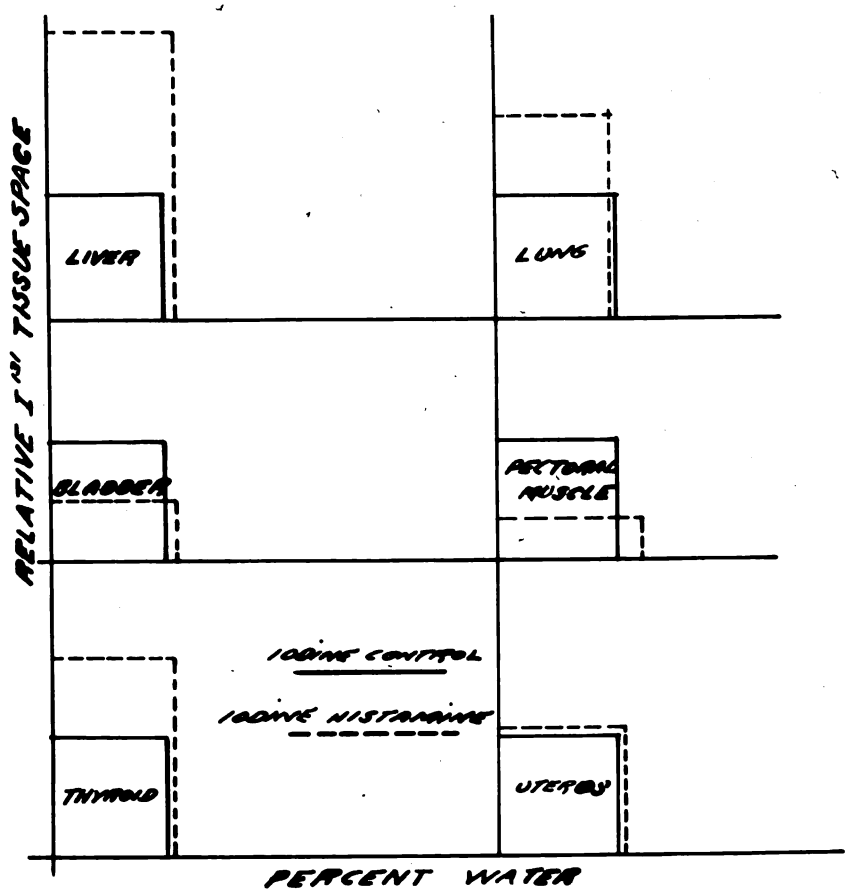


FIG. 3
RELATIVE CHANGES OF IODINE TISSUE SPACE AND WATER VOLUME

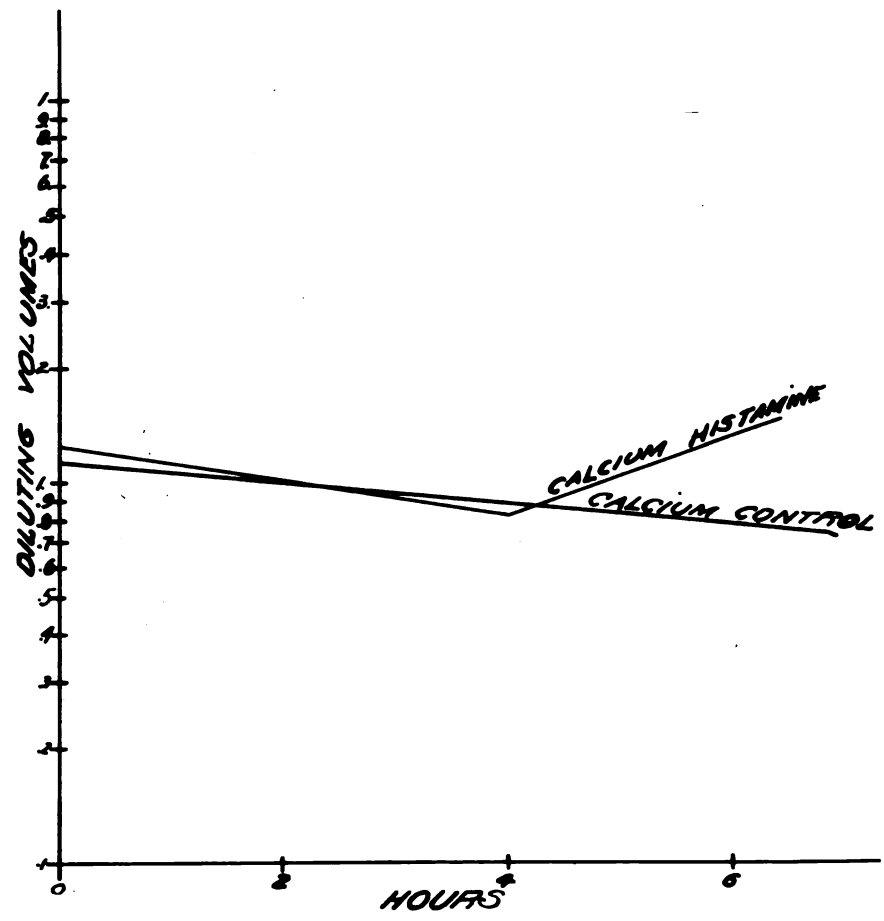


FIG 4

COMPOSITE CURVES SHOWING EFFECT OF HISTAMINE UPON THE VOLUME OF DILUTING FLUID FOR INJECTED CALCIUM.

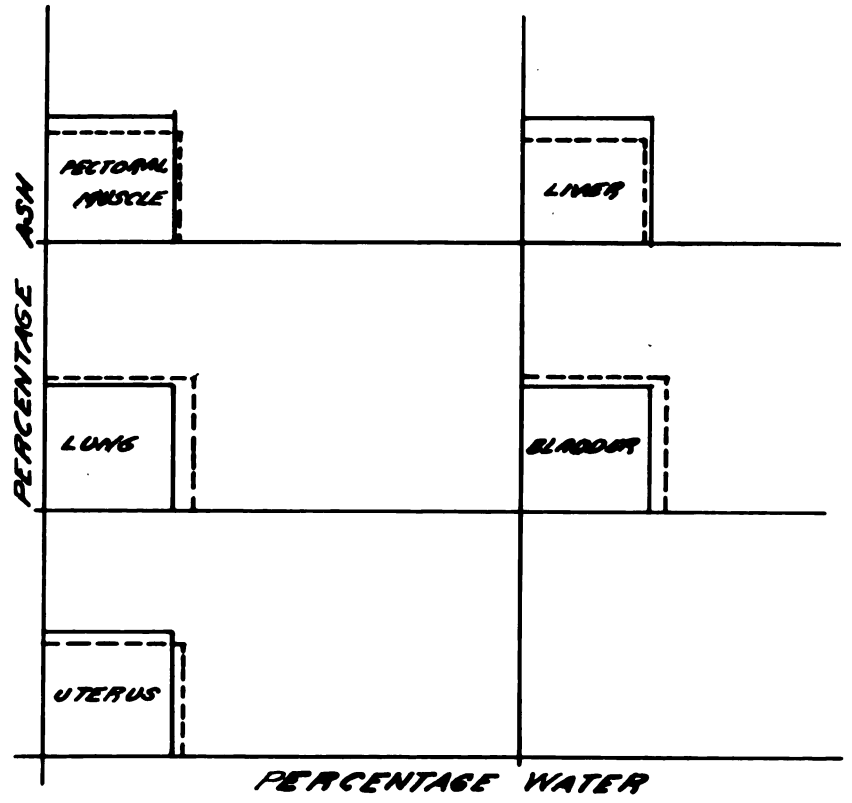


FIG. 5

RELATIVE WATER-MINERAL SHIFTS DURING HISTAMINE SHOCKS.

CALCIUM CONTROL ————

CALCIUM HISTAMINE - - - - - - -

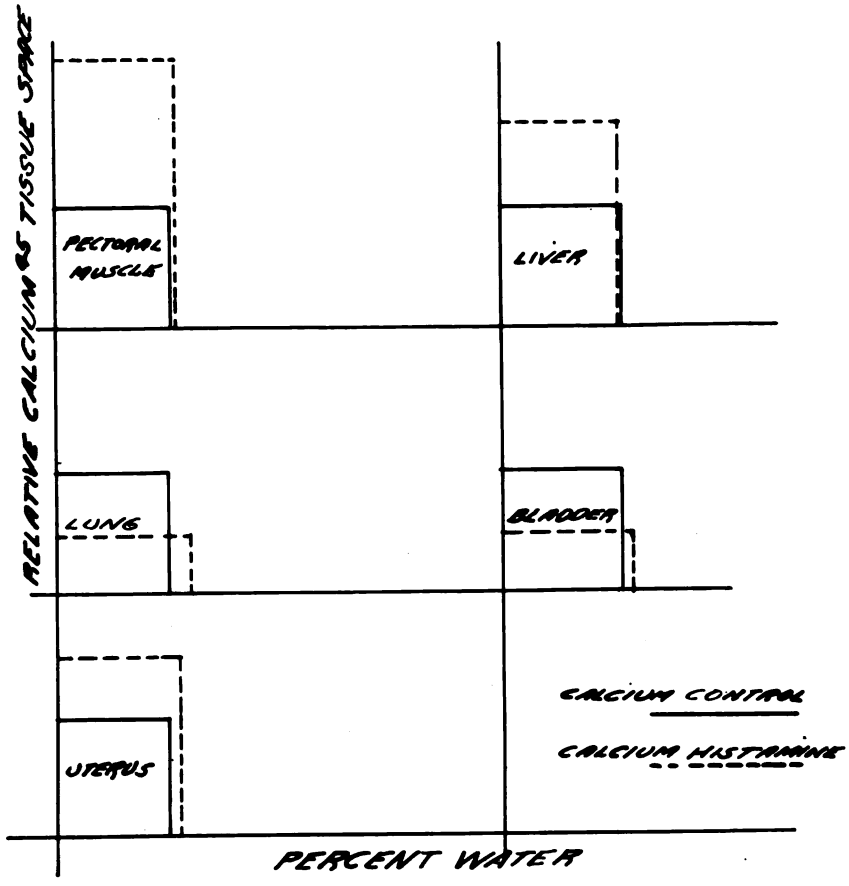


FIG 6

RELATIVE CHANGES OF CALCIUM TISSUE SPACE AND WATER VOLUME.

RESULTS

Iodine Experiment.

From the data on blood iodine radioactivity in Table II, the diluting fluid volumes were calculated (according to the equations on page 10). They indicate that the injected iodine was absorbed and diluted for a period of about four hours. After this the diluting volume became smaller progressively. However, during histamine shock, the iodine was further diluted five to six times above control values as shown in Figure 2.

The ash and water content of the tissues differed in control and histamine shocked animals, but all tissues did not vary in the same direction (Table I, Figure 1). Specifically the ash content of liver decreased, whereas the water content increased. A similar condition was noted for the bladder. The lung tissues demonstrated a contrary effect wherein the percentage ash increased, and the percentage water decreased. Pectoral muscle, thyroid gland, and uterus showed an increase in ash and water content.

The data in Table III showing the distribution of iodine radioactivity in the various tissues, both test and control, were used to calculate the fluid space in which the iodine was distributed within the tissues. As indicated in Table IV and Figure 3, experimental and control iodine space determinations increased as much as 200% in the case of liver. However, lung, thyroid and uterus showed a smaller increase, and bladder and pectoral muscle

actually underwent considerable reduction in the iodine space.

Calcium Experiment.

From the data in Table IV, the diluting volumes were calculated as for iodine (vide supra). These indicate apparently that absorption from the subcutaneous pocket at the injection site was slow. Thus control values show a decreasing diluting volume, although experimental values followed the controls closely. Upon injection of histamine and consequent shock, the diluting volume increased to about twice the control value (Figure 4).

The experimental and control percentages of water content varied similar to those in the iodine experiment (Table V, Figure 5). However, percentage ash varied in the opposite direction excepting lung and bladder which increased in both water and ash content. Pectoral muscle and uterus decreased in percentage ash, but water content increased. Liver, on the other hand, decreased both in ash and water content.

The calcium spaces (Table VIII) were calculated from the data in Table VII in the same manner as for iodine spaces. These were in no way related to the corresponding iodine spaces. However, as in the iodine experiment, the calcium spaces varied perceptibly among different tissues (Figure 6). Calcium space increased about four times in pectoral muscle and to a lesser degree in liver and uterus. In lung and bladder, the calcium spaces were markedly decreased.

In a single experiment as described in the section

above on methods, it was found that Ca^{45} and I^{131} failed to enter the erythrocytes either in control animals or in those subjected to histamine shock.

DISCUSSION

The phase determination experiments described above show that the injected isotopes of calcium and iodine do not enter the erythrocytes. Therefore, the intracellular fluid of the red cells plays no role in the dilution of these isotopes as discussed below.

The calculated diluting volumes of the radioactive iodine indicate rapid dilution or uptake of iodine from the extracellular phase, as shown in Figure 2. It should be noted that the slope of the plot of the diluting volume versus time (Figure 2) depended largely upon the rate of absorption of injected iodine from the subcutaneous injection site. Under the experimental conditions, however, it is further possible that there was a rapid dilution of the iodine by means of a shift of intracellular fluid to the extracellular phase. If this were true, a highly vascular organ which becomes engorged during shock, should show a great increase in iodine content during shock. This is apparently true since the iodine concentration in liver increased threefold (Table III). Another factor in determination of iodine concentration in extracellular fluid is its uptake by one or more tissues and dilution by intracellular water. Possibly, this occurred during shock in the case of thyroid, lung, liver and uterus (Table IV).

The plots of the diluting volumes of calcium versus time (Figure 4) indicate a slow but progressive concentration of calcium in the extracellular fluid of the organism. Under control conditions, this is probably due to the slow rate of absorption of the calcium at the site of the injection. Under experimental conditions the dilution increased as in the iodine experiment. Similar reasoning indicates that the dilution of the cal-

cium is due to both an increase in the diluting volume (extracellular fluid) and an uptake of calcium by specific tissues.

The question then arises: what is the role of calcium as compared to iodine during the shock phenomenon, and what is then their relationship to the distribution of water in the organism?

Definite shifts of water are indicated by the data in Tables I and V. These data are summarized in Table IX which contains the average values for total water content of the tissues. They indicate that under conditions of histamine shock, there is a general increase of total water content of tissues, with but two exceptions, namely lung and liver.

TABLE IX. WATER SHIFTS IN VARIOUS TISSUES UNDER THE CONDITION OF HISTAMINE SHOCK

	Iodine		Calcium	
Tissue	Histamine	Control	Histamine	Control
Liver	73.4%	72.3%	75.0%	75.9%
Lung	76.9	79.9	81.8	72.5
Bladder	83.2	82.6	88.0	82.8
Pectoral muscle	80.0	72.5	78.7	74.4
Thyroid	71.2	69.1		
Uterus	83.3	80.5	83.0	81.4
Av. H_2O	78.0	76.15	81.3	77.4

It is therefore established that the dilutions of iodine and calcium shown in Figures 2 and 4 are real as the result of a shift of water into the tissues. Since it is well known that movement of water into and out of the cells is accompanied by ionic shifts, some movement of calcium and iodine and possibly other ions might be expected to have occurred in these experiments. This may be indicated by the rate of exit of the isotopic ions from the blood (extracellular volume). The average rate of radiation from whole blood shows that calcium and iodine leave the blood at the same rate under shock conditions. Data showing this are summarized in Table X and accompanying ratios.

TABLE X MEAN RADIATION COUNTS IN WHOLE BLOOD FOR CALCIUM AND IODINE

	Iodine-131		Calcium-45	
	Average Blood Counts		Average Blood Counts	
Time	Histamine	Control	Histamine	Control
2 Hours	15.65 *	11.96	10.70	12.69
4 Hours	7.32	6.96	13.04	11.32
6 Hours	5.13	6.37	9.30	13.25
Av. 2, and 4 Hours	10.89	9.46	11.87	12.01

* Counts per ml./sec.

From Table X the following ratios show the compar-

able rates of diffusion of iodine and calcium under control and experimental conditions:

$\frac{5.13}{10.89} = 0.47$ for iodine and histamine $\frac{6.37}{9.46} = 0.67$ for iodine control

$\frac{9.30}{11.87} = 0.78$ for calcium and histamine $\frac{13.25}{12.01} = 1.10$

Therefore $\frac{.67}{.47} = 1.426$ for the iodine experiment

Therefore $\frac{1.10}{.78} = 1.410$ for the calcium experiment

The last two ratios above show that both iodine and calcium leave the blood (extracellular fluid) at about the same rate.

In view of the fact that water appears to enter the tissues and that calcium and iodine seem to leave the extracellular fluid, the tissue content of these isotopes during shock becomes of interest.

From the data in Tables III and VII, the general trend in redistribution of calcium and iodine can be seen. Table XI summarizes the mean changes in concentrations of isotopes in the various fresh tissues.

TABLE XI SHIFT OF ISOTOPES DURING HISTAMINE SHOCK

Tissue	Iodine		Calcium	
	Histamine	Control	Histamine	Control
	Counts per second per gm. tissue			
Liver	4.69	1.53	19.00	16.23
Lung	5.30	3.65	15.15	37.92
Bladder	4.35	5.96	17.99	19.70

TABLE XI (Continued) SHIFT OF ISOTOPES DURING HISTAMINE SHOCK

	Iodine		Calcium	
	Histamine	Control	Histamine	Control
	Counts per second per gm. tissue			
Pectoral muscle	1.72	1.03	33.80	6.81
Thyroid	139.48	114.22		
Uterus	6.60	7.15		

Figures 3 and 6 depict graphically a resume of the data on iodine and water, and calcium and water, respectively. These show that apparently in shock, with the notable exception of pectoral muscle, water and calcium move in opposite directions. That is to say, wherever calcium enters a tissue the total water content decreases and vice versa. No such conclusion can be drawn from the iodine data.

Considering the total ash content of the tissue, it appears as if there was some sort of change in this material as well as tissue water in the shock experiments. The extent of this shift can be seen in Table XII which summarizes data in Tables II and VII.

TABLE XII REDISTRIBUTION OF ASH DURING HISTAMINE SHOCK IN IODINE AND CALCIUM EXPERIMENTS

Tissue	Iodine % Ash		Calcium % Ash	
	Histamine	Control	Histamine	Control
Liver	5.8	6.2	5.7	7.3
Lung	11.5	8.2	7.0	6.8

TABLE XII (Continued) REDISTRIBUTION OF ASH DURING HISTAMINE SHOCK IN IODINE AND CALCIUM EXPERIMENTS

	Iodine % Ash		Calcium % Ash	
Tissue	Histamine	Control	Histamine	Control
Bladder	11.1	12.0	7.6	5.8
Pectoral muscle	8.6	8.0	5.7	6.8
Thyroid	9.1	6.7		
Uterus	13.9	5.0	6.5	7.2
Av.	10.0	7.7	6.5	6.8

From Tables XI and XII it can be seen that calcium and ash move in the opposite directions. Therefore, it can also be said that water and ash accompany each other under shock conditions. In the case of iodine, however, the figures indicate with few exceptions iodine, ash and water travel in the same directions.

In general the tissue spaces as calculated on page 11 (Figures 3 and 6) represent a dynamic picture of the previous discussion. However, from the standpoint of actual space, it is to be noted that the standard method of calculation of space as given by Mannery and Haege (1941) cannot apply in its entirety

in this problem. It is obvious that in any biological phenomenon where permeability is altered, the Gibbs-Donnan ratio as given in the calculations will not apply. Similarly, in any investigation where water shifts are as extensive as those noted above, no standard fraction of water content can be given for plasma. Nevertheless, even though these aforesaid constants do have errors, if they are constant, the calculated tissue spaces will be relatively correct.

The evidence presented indicates that there is a shift of calcium from lung and bladder, whereas calcium enters pectoral muscle. If these tissues can be considered representative of smooth and striated muscle respectively, it is probably quite significant that calcium should leave a tissue such as lung which is so prominently involved in the cause of death in histamine shock in the guinea pig. Moreover, there is some indication that mineral ash may increase in smooth muscle during shock of this type.

SUMMARY AND CONCLUSIONS

1. Two experimental groups of histamine-shocked (2.75 mg. histamine acid phosphate per kilogram body weight), which were previously injected with radioactive calcium 45 and iodine 131 respectively, were compared to two control groups of unshocked guinea pigs with respect to total extracellular volume.

For measurements of water, and isotope content 1 ml. blood samples were secured by heart puncture at 2, 4 and 6 hours following isotope injection. Experimental blood samples were taken shortly after injection of histamine at 6 hours. All animals were then sacrificed and samples of liver, lung, pectoralis muscle, bladder, uterus, and in some instances, thyroid tissue were obtained for isotope, water and ash determinations.

2. The data indicate that there was a tendency for body water to shift into the tissues during histamine shock.

3. Some evidence is presented to indicate that in shock tissue calcium and ash content varied in opposite directions.

4. Ionic iodine, not having been previously implicated in this type of shock, was used for comparison with calcium. Contrary to calcium, tissue iodine was found to vary in the same direction as tissue ash content.

5. It was shown that during shock both calcium and iodine leave the blood at the same rate.

6. It is considered significant that calcium space decreased in smooth muscle containing tissues, i.e. lung and bladder, whereas calcium space increased in striated since severe smooth muscle spasm is so characteristic in guinea pig histamine shock, muscle, i.e. pectoral muscle. Concomitantly ash content of striated muscle appeared to decrease whereas ash in smooth muscle increased.

7. The data presented do not justify a firm conclusion based upon a comparison of iodine and calcium tissue space. However, if any trend is indicated it is that iodine space increases in lung and decreases in pectoral muscle contrary to the calcium data.

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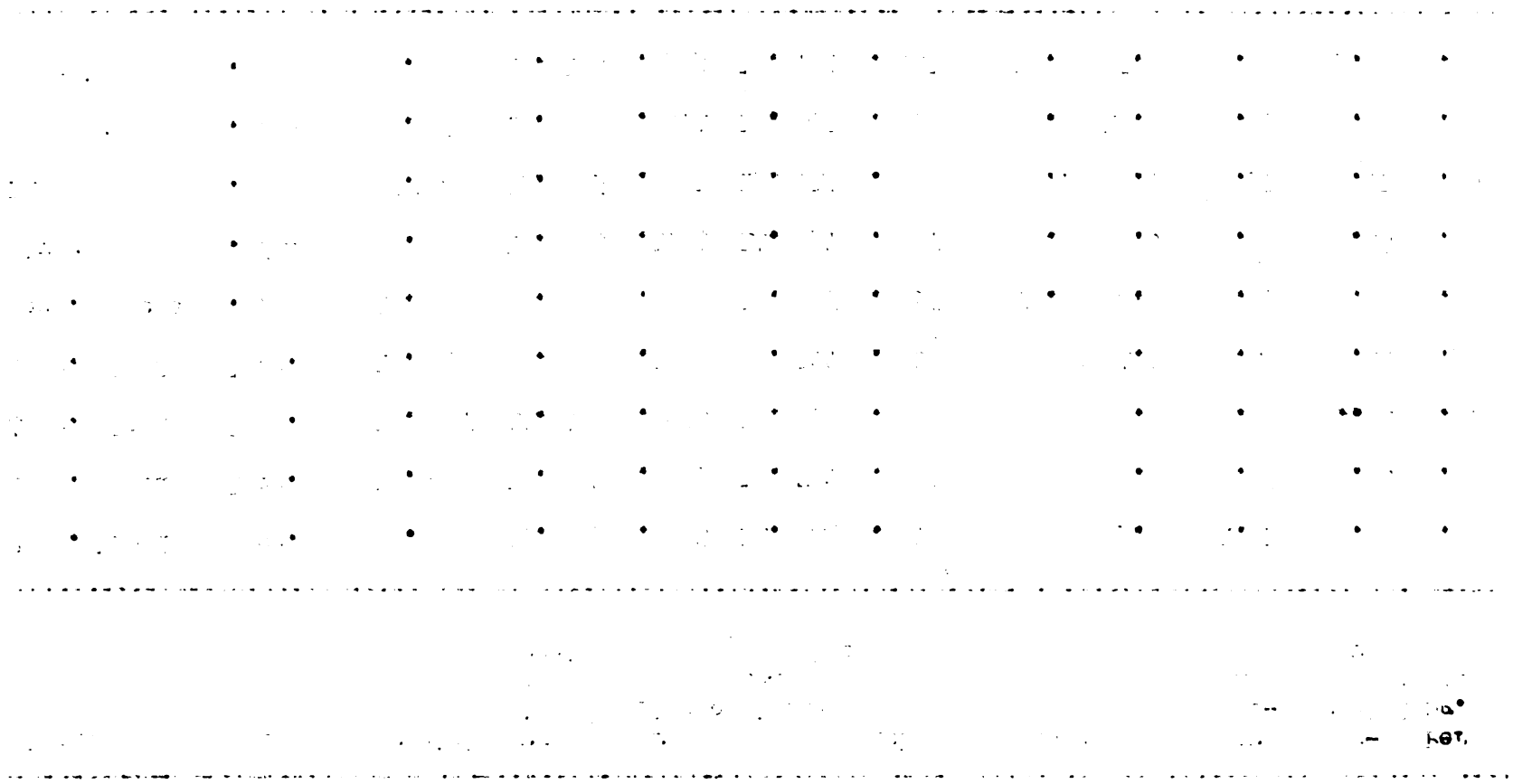
IODINE CONTROL

Animal No.1
Body Weight: 1097 Grams

		*Weights					Percentage		Counts		
Tissue	Wet	Crucible	Dry Tissue and Crucible	Dry Tissue	Ash Tissue and Crucible	Ash	Water	Ash	Per Second	Corrected Counts Per Second	Per Mg. Ash
2 Hr. blood	1 cc.	14.8565	15.0663	.2098	14.8644	.0079		3.8	1.81	19.09	2.52
2 Hr. blood	1 cc.	14.6628	14.8717	.2089	14.6707	.0079		3.8	.36	3.96	.50
4 Hr. blood	1 cc.	14.6367	14.8293	.1926	14.6456	.0089		4.6	5.94	5.94	.67
6 Hr. blood	1 cc.	15.0095	15.1923	.1828	15.0177	.0082		4.5	2.62	2.62	.32
Pect. Muscle	.2958	14.9180	14.9848	.0668	14.9235	.0055	77.4	8.2	.09	.09	.02
Liver	.6792	14.8740	15.0444	.1704	14.8837	.0097	74.9	5.6	.06	.66	.07
Lung	.3108	15.2664	15.3265	.0601	15.2714	.0050	80.7	8.3	.31	.31	.06
Bladder	.1740	15.0617	15.0891	.0274	15.0651	.0034	84.3	12.4	.23	.23	.07
Thyroid	.1330	14.8733	14.9126	.0393	14.8754	.0021	70.5	5.3	3.09	3.09	1.47

*Large crucible (geometry)
All counts are corrected for background



IODINE CONTROL

Animal No.2
Body Weight: 977 Grams

Tissue	*Weights Wet	Crucible	Dry Tissue and Crucible	Dry Tissue	Ash Tissue and Crucible	Ash	Percentage Water	Ash	Counts Per Second	Corrected Counts Per Second	Per Mg. Ash
2 Hr. blood	1 cc.	15.1560	15.3482	.1922	15.1635	.0075		3.9	.67	7.37	.98
4 Hr. blood	1 cc.	15.0256	15.2444	.2188	15.0348	.0092		4.2	3.85	3.85	.42
Pct. Muscle	.3094	15.7733	15.8447	.0714	15.7796	.0063		8.8	.04	.04	.01
Liver	.4462	14.5424	14.6495	.1071	14.5497	.0073	76.0	6.8	.43	.43	.06
Lung	.4358	14.2719	14.3646	.0927	14.2784	.0065	78.7	7.0	.47	.47	.07
Bladder	.1258	15.1992	15.2188	.0196	15.2022	.0030	84.4	15.3	1.47	1.47	.49
Thyroid	.1106	15.6462	15.6780	.0318	15.6484	.0022	71.2	6.9	1.67	1.67	.76

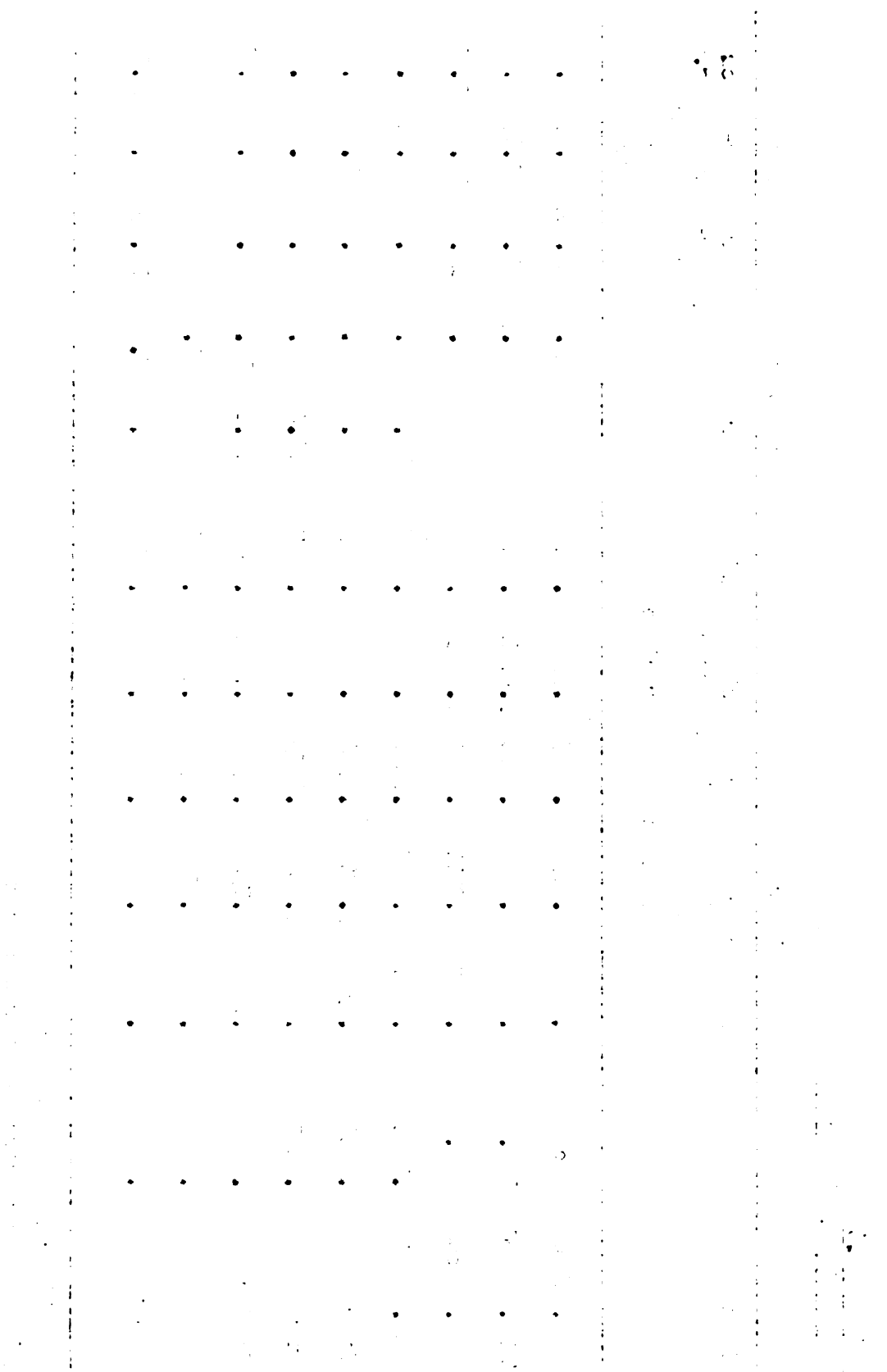
*Large crucibles (geometry)
All counts are corrected for background

IODINE CONTROL

Animal No.6
Body Weight: 580 Grams.

Tissue	Weights*					Percentage		Counts		
	Wet	Crucible	Dry Tissue and Crucible	Dry Tissue	Ash Tissue and Crucible	Ash	Water	Ash	Per Second	Corrected Counts Per Second	Per Mg. Ash
2 Hr. blood	1cc.	5.9515	6.1734	.2219	5.9594	.0079		3.6	10.69	10.69	1.35
4 Hr. blood	1cc.	5.6638	5.8508	.1870	5.6711	.0073		3.9	11.29	11.29	1.55
6 Hr. blood	1cc.	6.2123	6.3977	.1854	6.2183	.0060		3.2	10.25	10.25	1.71
Pect.Muscle	.1420	6.2887	6.3314	.0427	6.2934	.0047	70.0	11.0	.29	.29	.06
Liver	.3220	5.8395	5.9379	.0984	5.8468	.0073	69.5	7.4	1.04	1.04	.14
Lung	.2280	5.5029	5.5682	.0653	5.5096	.0067	71.4	10.2	1.91	1.91	.29
Bladder	.1530	5.7000	5.7322	.0322	5.7041	.0041	79.0	12.7	.85	.85	.21
Uterus	.1864	6.1190	6.3166	.1976	6.1266	.0076		3.8			
Thyroid	.0696	5.7552	5.7778	.0226	5.7565	.0013	67.6	5.8	18.39	18.39	14.15

* Small crucibles (geometry)
All counts are corrected for background

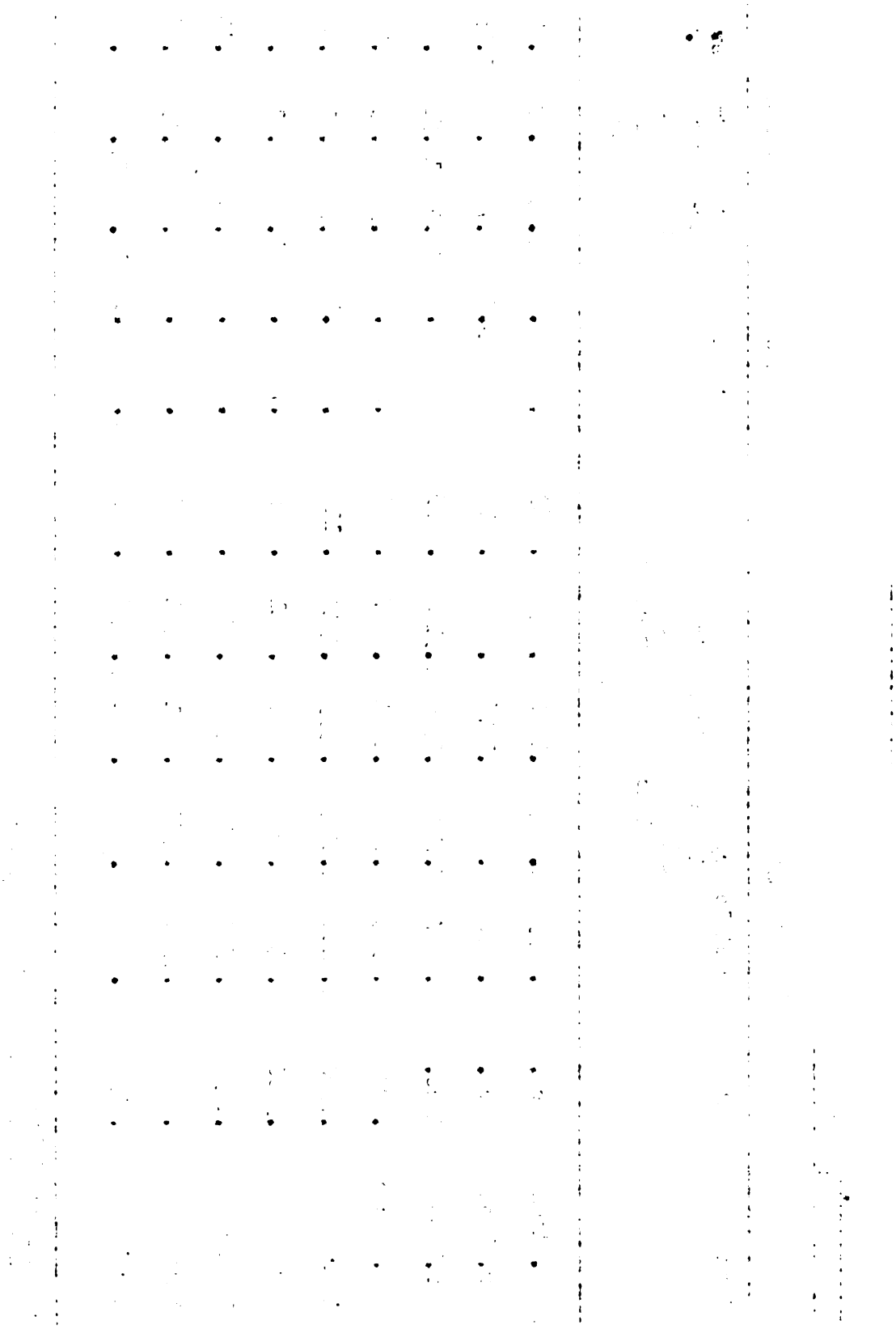


IODINE CONTROL

Animal No.7
Body Weight: 615 Grams

	Weights*					Percentage		Counts		
Tissue	Wet	Crucible	Dry Tissue and Crucible	Dry Tissue	Ash Tissue and Crucible	Ash	Water	Ash	Per Second	Corrected Counts Per Second	Per Mg. Ash
2 Hr.blood	1 cc.	6.0833	6.2653	.1820	6.0896	.0063		3.5	9.89	9.89	1.57
4 Hr.blood	1 cc.	6.1190	6.3166	.1976	6.1266	.0076		3.8	8.75	8.75	1.15
6 Hr.blood	1 cc.	5.5127	5.7107	.1980	5.5182	.0055		2.8	12.56	12.56	2.28
Pect.Muscle	.2008	6.0584	6.1188	.0604	6.0607	.0023	70.0	3.8	.33	.33	.14
Liver	.7500	6.0222	6.2555	.2333	6.0340	.0118	68.9	5.1	.71	.71	.06
Lung	.3600	6.0486	6.1253	.0767	6.0541	.0055	88.7	7.2	1.51	1.51	.27
Bladder	.1836	5.8555	5.8871	.0316	5.8579	.0024	82.8	7.6	.97	.97	.40
Uterus	.2000	6.1920	6.2311	.0391	6.1944	.0024	80.5	6.1	1.43	1.43	.56
Thyroid	.1130	5.8902	5.9274	.0372	5.8935	.0033	67.1	8.9	17.44	17.44	5.28

* Small crucibles (geometry)
All counts are corrected for background

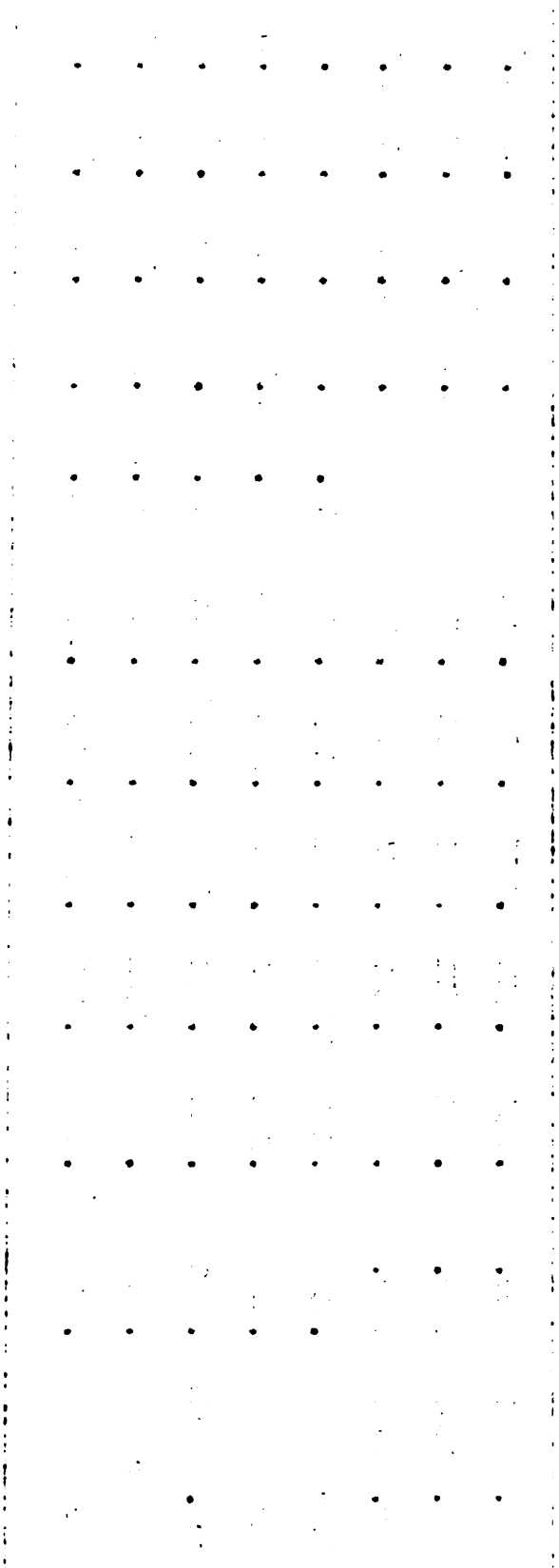


CALCIUM CONTROL

Animal No.2
Body Weight: 610 Grams

		Weights					Percentage		Counts		
Tissue	Wet	Crucible	Dry Tissue and Crucible	Dry Tissue	Ash Tissue and Crucible	Ash	Water	Ash	Per Second	Corrected Counts Per Second	Per Mg. Ash
2 Hr. blood	1 cc.	5.6854	5.8372	.1518	5.6916	.0062		4.1	7.05	7.05	1.14
4 Hr. blood	1 cc.	6.2981	6.4671	.1690	6.3054	.0073		4.3	6.70	6.70	.92
6 Hr. blood	1 cc.	6.2143	6.3687	.1544	6.2203	.0060		3.9	6.97	6.97	1.16
Liver	.5480	5.5707	5.7188	.1481	5.5785	.0078	73.0	5.3	3.99	3.99	.51
Lung	.3220	5.7776	5.8464	.0688	5.7826	.0050	78.6	7.3	2.83	2.83	.57
Pect. Muscle	.4822	6.2922	6.3930	.1008	6.2989	.0067	79.1	6.6	2.96	2.96	.44
Bladder	.1472	5.7670	5.7924	.0254	5.7686	.0016	82.7	6.3	1.12	1.12	.70
Uterus	.2872	5.0252	5.0781	.0529	5.0285	.0033	81.6	6.2	2.17	2.17	.66

All counts are corrected for background

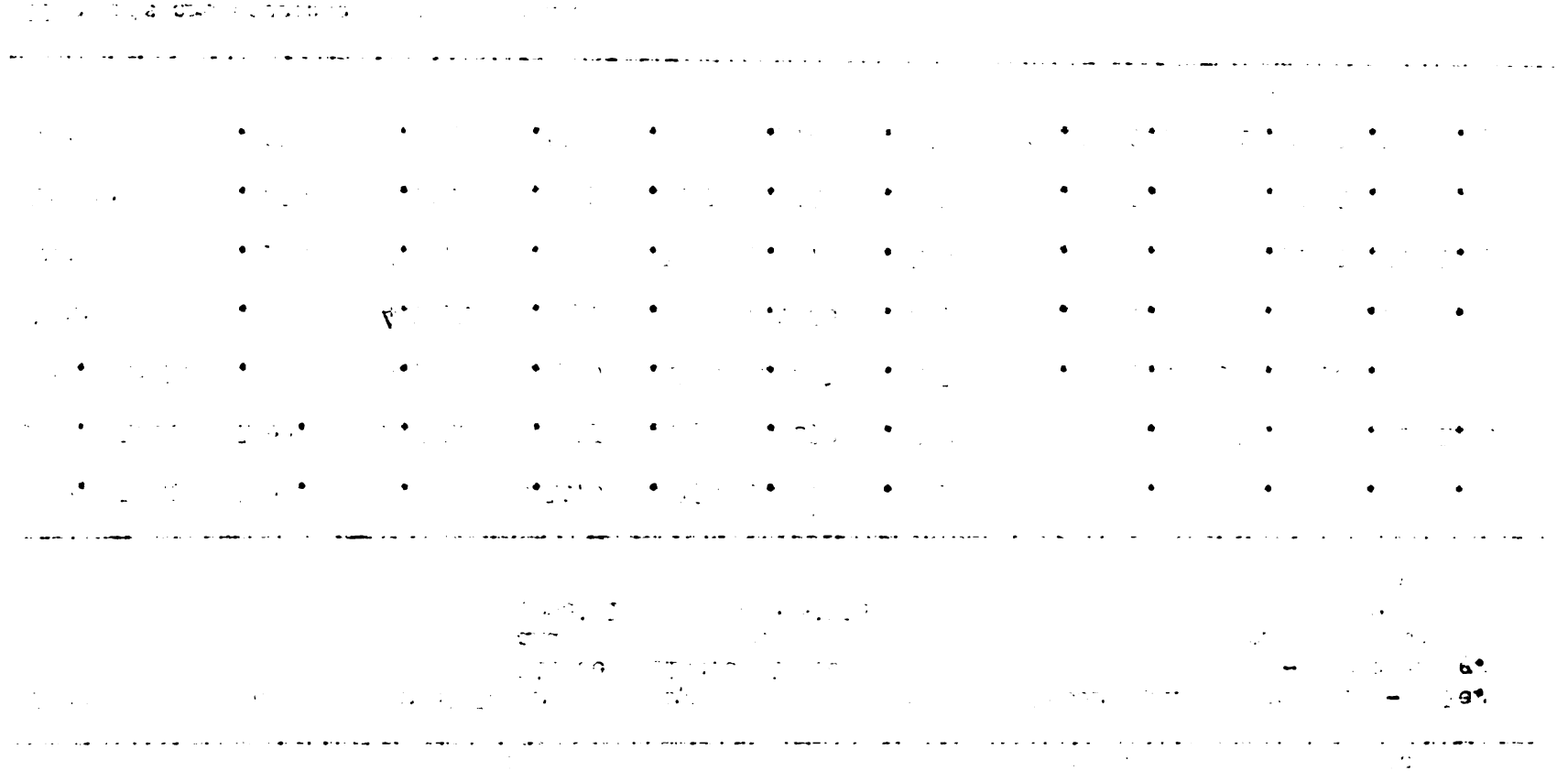


CALCIUM CONTROL

Animal No.7
Body Weight: 925 Grams

	Weights						Percentage		Counts		
Tissue	Wet	Crucible	Dry Tissue and Crucible	Dry Tissue	Ash Tissue and Crucible	Ash	Water	Ash	Per Second	Corrected Counts Per Second	Per Mg. Ash
2 Hr. blood	1 cc.	5.9780	6.1976	.2196	5.9873	.0093		4.2	20.34	20.34	2.19
4 Hr. blood	1 cc.	4.5810	4.7805	.1995	4.5890	.0080		4.0	11.41	11.41	1.42
Pect. Muscle	.5260	4.9150	5.0326	.1176	4.9261	.0111	77.6	9.4	580.83	580.83	
Liver	.9637	4.7651	5.0637	.2986	4.7806	.0209	69.0	7.0	29.78	29.78	1.42
Lung	.3374	5.5872	5.7397	.1525	5.5972	.0100	54.8	6.5	18.52	18.52	1.85
Bladder	.3416	6.0442	6.1043	.0601	6.0449	.0007	82.4	1.2	21.55	21.55	30.79
Uterus	.2918	6.3723	6.4322	.0599	6.3769	.0046	79.5	7.7	12.12	12.12	2.63

All counts are corrected for background

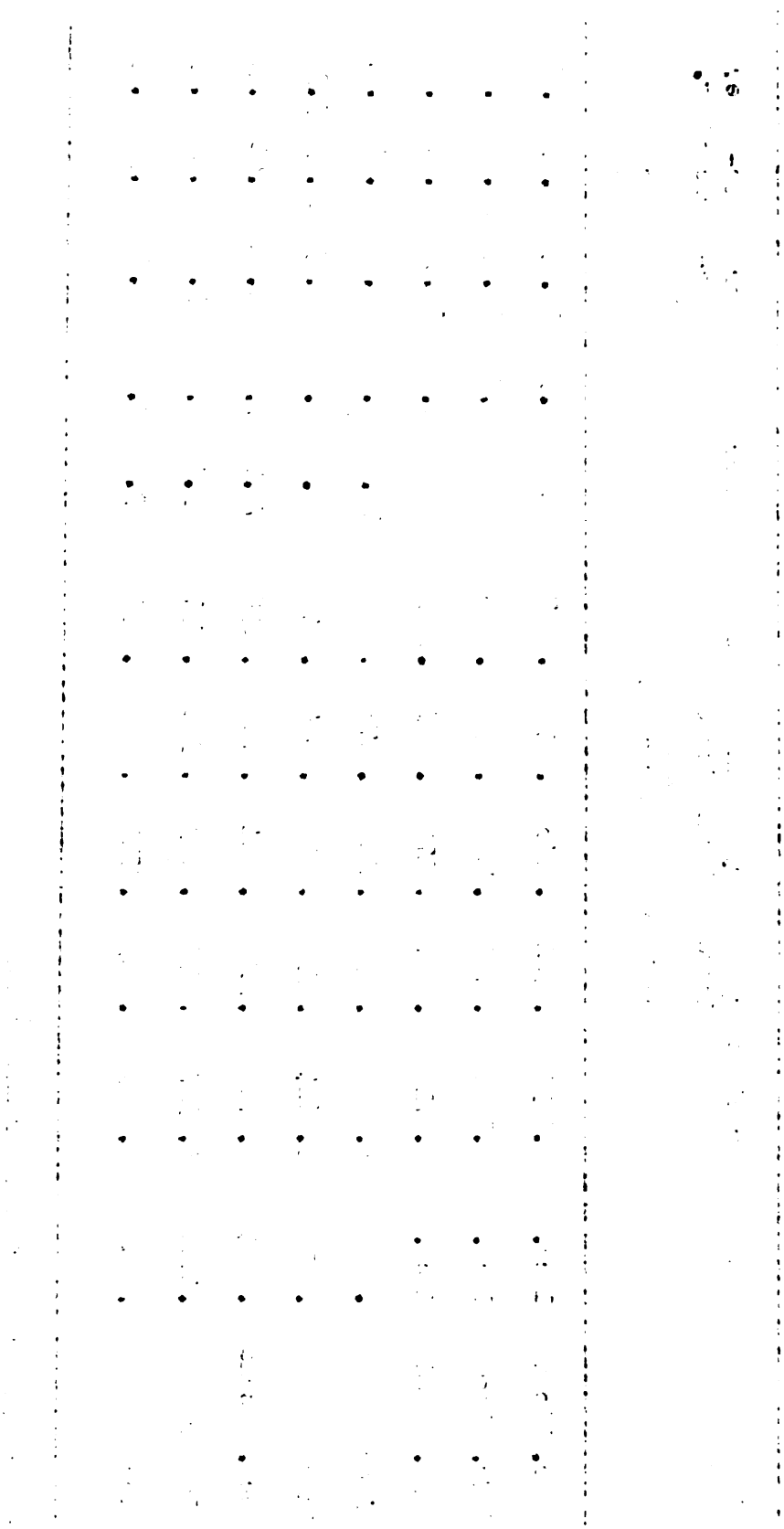


CALCIUM CONTROL

Animal No.1
Body Weight: 700 Grams

	Weights						Percentage		Counts		
Tissue	Wet	Crucible	Dry Tissue and Crucible	Dry Tissue	Ash Tissue and Crucible	Ash	Water	Ash	Per Second	Corrected Counts Per Second	Per Mg. Ash
2 Hr. blood	1 cc.	6.1039	6.2984	.1945	6.1096	.0057		2.9	10.69	10.69	1.88
4 Hr. blood	1 cc.	6.1582	6.3784	.2202	6.1638	.0056		2.5	15.85	15.85	2.83
6 Hr. blood	1 cc.	6.3712	6.5887	.2175	6.3775	.0063		2.9	19.52	19.52	3.10
Liver	.4094	5.8008	5.9130	.1122	5.8073	.0065	72.6	5.8	4.34	4.34	.67
Lung	.4718	5.9634	6.0553	.0939	5.9684	.0070	80.1	7.5	23.63	23.63	3.38
Pect. Muscle	.2464	5.6722	5.7246	.0524	5.6760	.0038	78.7	7.3	1.84	1.84	.48
Bladder	.4222	5.8347	5.9050	.0703	5.8384	.0037	83.3	5.3	13.42	13.42	3.63
Uterus	.3062	6.0869	6.1383	.0514	6.0908	.0039	83.2	7.6	5.59	5.59	1.43

All counts are corrected for background

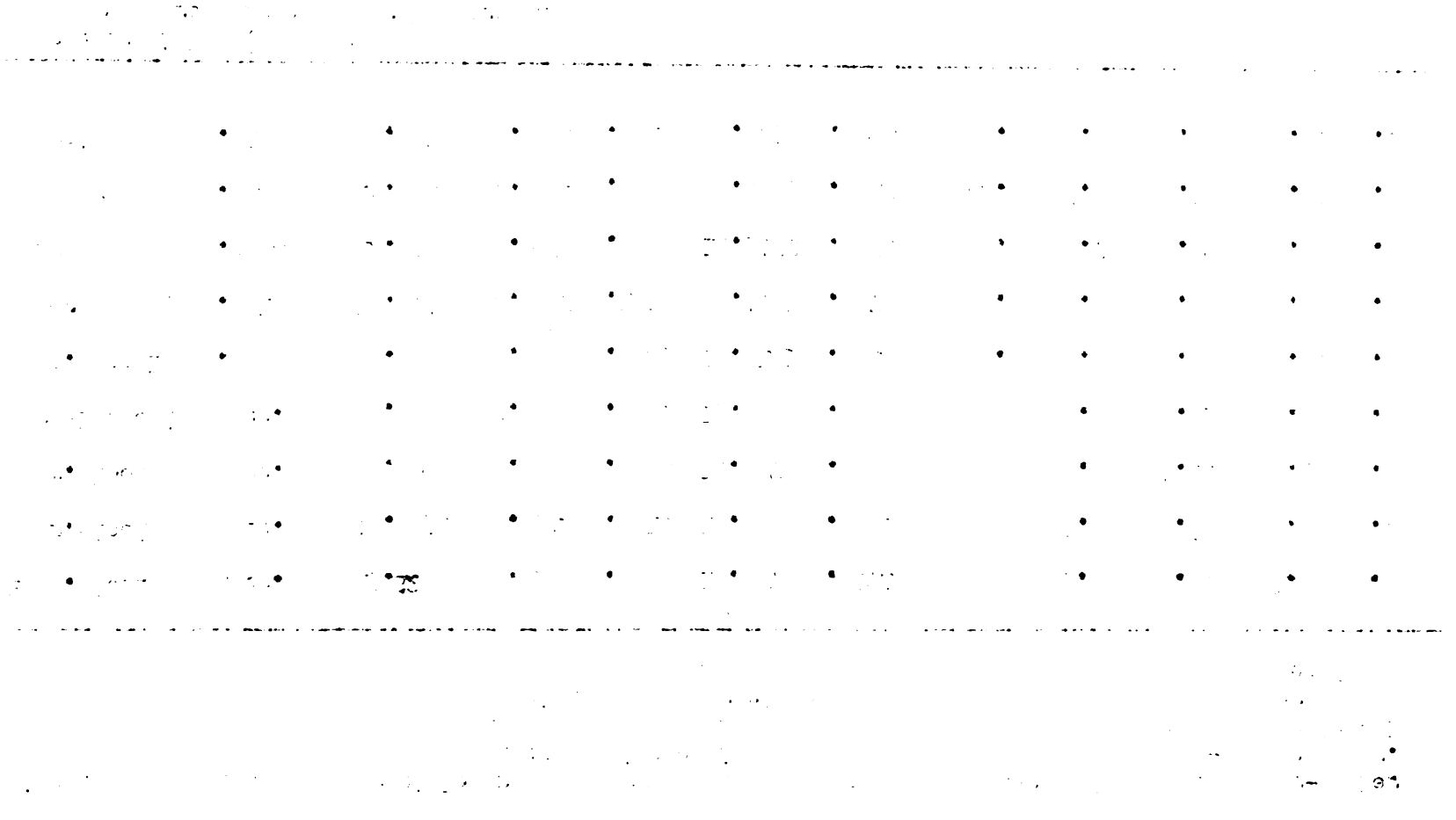


IODINE AND HISTAMINE

Animal No.3
Body Weight: 982 Grams

		*Weights					Percentage		Counts		
Tissue	Wet	Crucible	Dry Tissue and Crucible	Dry Tissue	Ash Tissue and Crucible	Ash	Water	Ash	Per Second	Corrected Counts Per Second	Per Mg. Ash
2 Hr.blood	1 cc.	15.8299	16.0399	.2100	15.8373	.0074		3.5	1.92	21.23	2.87
4 Hr.blood	1 cc.	16.0832	16.3344	.2512	16.0922	.0090		3.6	.65	7.15	.79
6 Hr.blood	1 cc.	14.8002	15.0308	.2306	14.8070	.0068		2.9	.22	2.42	.36
Portal blood	1 cc.	15.4026	15.4923	.0897	15.4069	.0048		5.4	.43	4.73	.99
Pect. Muscle	.2402	14.6075	14.6604	.0529	14.6123	.0048	78.0	9.1	.00	.00	.00
Liver	.4318	14.4749	14.5964	.1215	14.4821	.0072	71.9	5.9	.10	.10	.01
Lung	.2924	14.5400	14.6049	.0649	14.5450	.0050	77.9	7.7	.29	.29	.06
Bladder	.2840	15.2346	15.2831	.0485	15.2389	.0043	82.9	8.8	.14	.14	.03
Thyroid	.1120	15.8017	15.8327	.0310	15.8053	.0036	72.4	11.6	5.87	5.87	1.63

*Large crucibles (geometry)
All counts are corrected for background

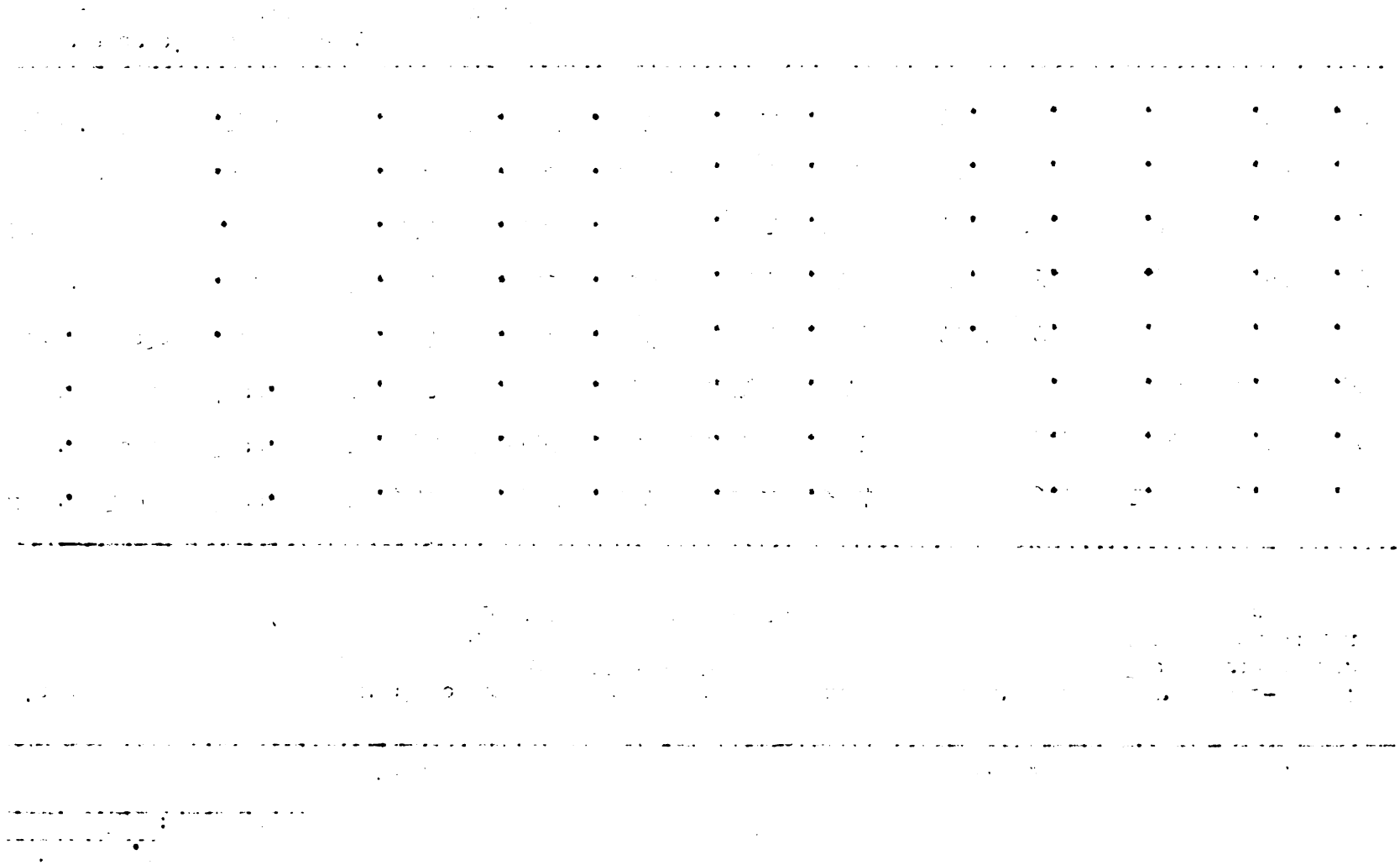


IODINE AND HISTAMINE

Animal No.4
Body Weight: 885 Grams

Tissue	*Weights					Percentage		Counts		
	Wet	Crucible	Dry Tissue and Crucible	Dry Tissue	Ash Tissue and Crucible	Ash	Water	Ash	Per Second	Corrected Counts Per Second	Per Mg. Ash
2 Hr. blood	1 cc.	15.4578	15.6305	.1727	15.4642	.0064		3.7	1.22	13.42	2.09
4 Hr. blood	1 cc.	14.8032	15.0054	.2022	14.8103	.0071		3.5	.50	5.50	.77
6 Hr. blood	1 cc.	14.5061	14.7153	.2092	14.5136	.0075		3.6	.26	2.86	.38
Pect. Muscle	.4084	15.1567	15.2492	.0925	15.1636	.0069	77.4	7.5	.00	.00	.00
Liver	.8758	14.8419	15.0353	.1934	14.8527	.0108	78.0	5.5	.55	.55	.05
Lung	.1966	14.8519	14.8928	.0409	14.8571	.0052	79.2	12.7	.18	.18	.03
Bladder	.2450	16.0046	16.0423	.0377	16.0086	.0040	84.6	10.6	.00	.00	.00
Thyroid	.1356	15.5088	15.5465	.0377	15.5114	.0026	72.2	6.9	5.12	5.12	1.97

* Large crucibles (geometry)
All counts are corrected for background

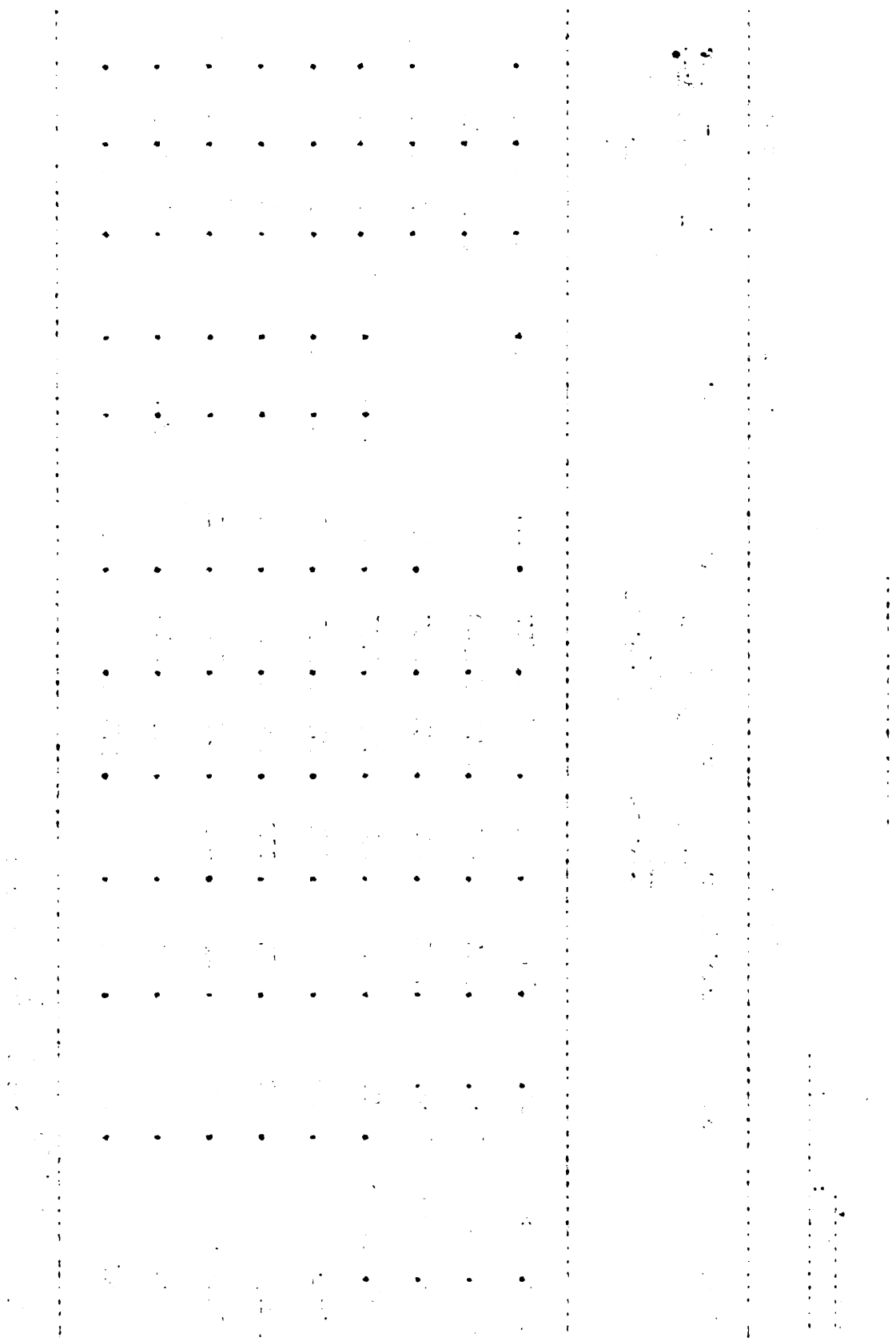


IODINE AND HISTAMINE

Animal No.8
Body Weight: 670 Grams

	*Weights						Percentage		Counts		
Tissue	Wet	Crucible	Dry Tissue and Crucible	Dry Tissue	Ash Tissue and Crucible	Ash	Water	Ash	Per Second	Corrected Counts Per Second	Per Mg. Ash
2 Hr. blood	1 cc.	6.1600	6.3625	.2025	6.1677	.0077		3.8	12.29	12.29	1.60
4 Hr. blood	1 cc.	5.9677	6.0651	.0974	5.9320				6.42	6.42	
6 Hr. blood	1 cc.	5.7801	6.0042	.2241	5.7857	.0056			8.37	8.37	1.49
Pect. Muscle	.3270	6.0676	6.1459	.0783	6.0735	.0059	76.1	7.5	.37	.37	.06
Liver	.3460	5.8980	5.9953	.0973	5.9031	.0051	71.8	5.2	.39	4.88	.96
Lung	.2580	5.8417	5.9119	.0702	5.8484	.0067	72.8	9.5	2.04	2.04	.30
Bladder	.2700	5.5843	5.6263	.0420	5.5884	.0041	84.4	9.7	.23	2.88	.70
Uterus	.2800	6.1805	6.2264	.0459	6.1870	.0065	83.7	14.2	.14	1.75	.27
Thyroid	.1260	5.9459	5.9829	.0370	5.9490	.0031	70.7	8.3	2.70	33.75	10.89

* Small crucibles (geometry)
All counts are corrected for background

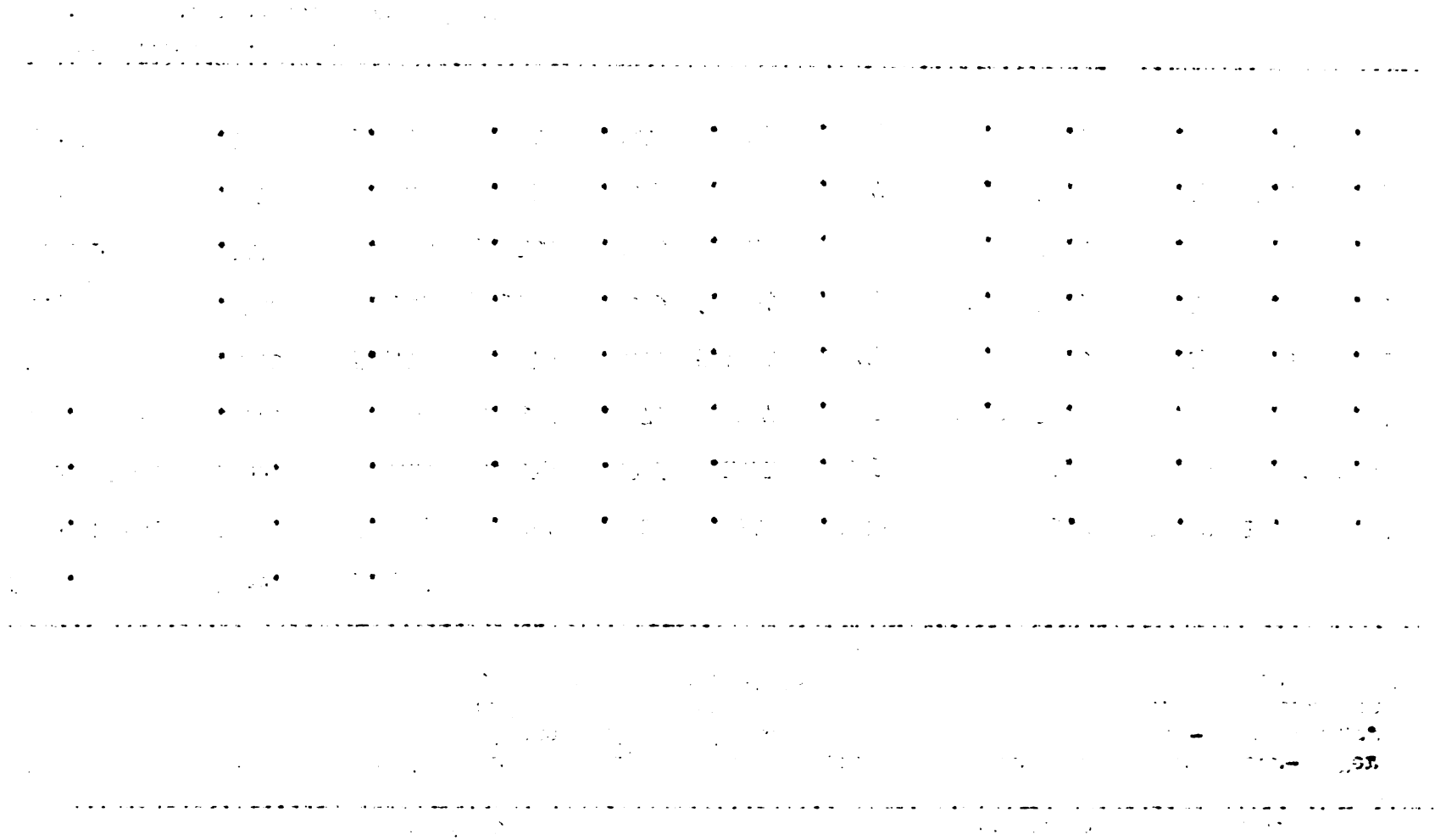


IODINE AND HISTAMINE

Animal No.10
Body Weight:710 Grams

	*Weights						Percentage		Counts		
Tissue	Wet	Crucible	Dry Tissue and Crucible	Dry Tissue	Ash Tissue and Crucible	Ash	Water	Ash	Per Second	Corrected Counts Per Second	Per Mg. Ash
2 Hr. blood	1 cc.	5.8361									
4 Hr. blood	1 cc.	6.0669	6.2754	.2085	6.0749	.0080		3.8	10.22	10.22	1.28
6 Hr. blood	1 cc.	6.1218	6.3213	.1995	6.1281	.0063		3.2	6.85	6.85	1.09
Pect. Muscle	.1740	6.2068	6.2447	.0379	6.2107	.0039	88.3	10.3	.08	1.00	.28
Liver	.4280	5.4775	5.5975	.1200	5.4854	.0079	72.0	6.6	.13	1.63	.21
Lung	.1100	6.3325	6.3570	.0245	6.3364	.0039	77.8	15.9	.10	1.25	.32
Bladder	.1440	5.8886	5.9162	.0276	5.8928	.0042	80.8	15.2	.08	1.00	.24
Uterus	.2470	6.1627	6.2050	.0433	6.1680	.0057	82.9	13.5	.13	1.63	.29
Thyroid	.1140	5.9695	6.0044	.0349	5.9729	.0034	69.4	9.7	22.79	22.79	6.70

* Small crucible (geometry)
All counts are corrected for background

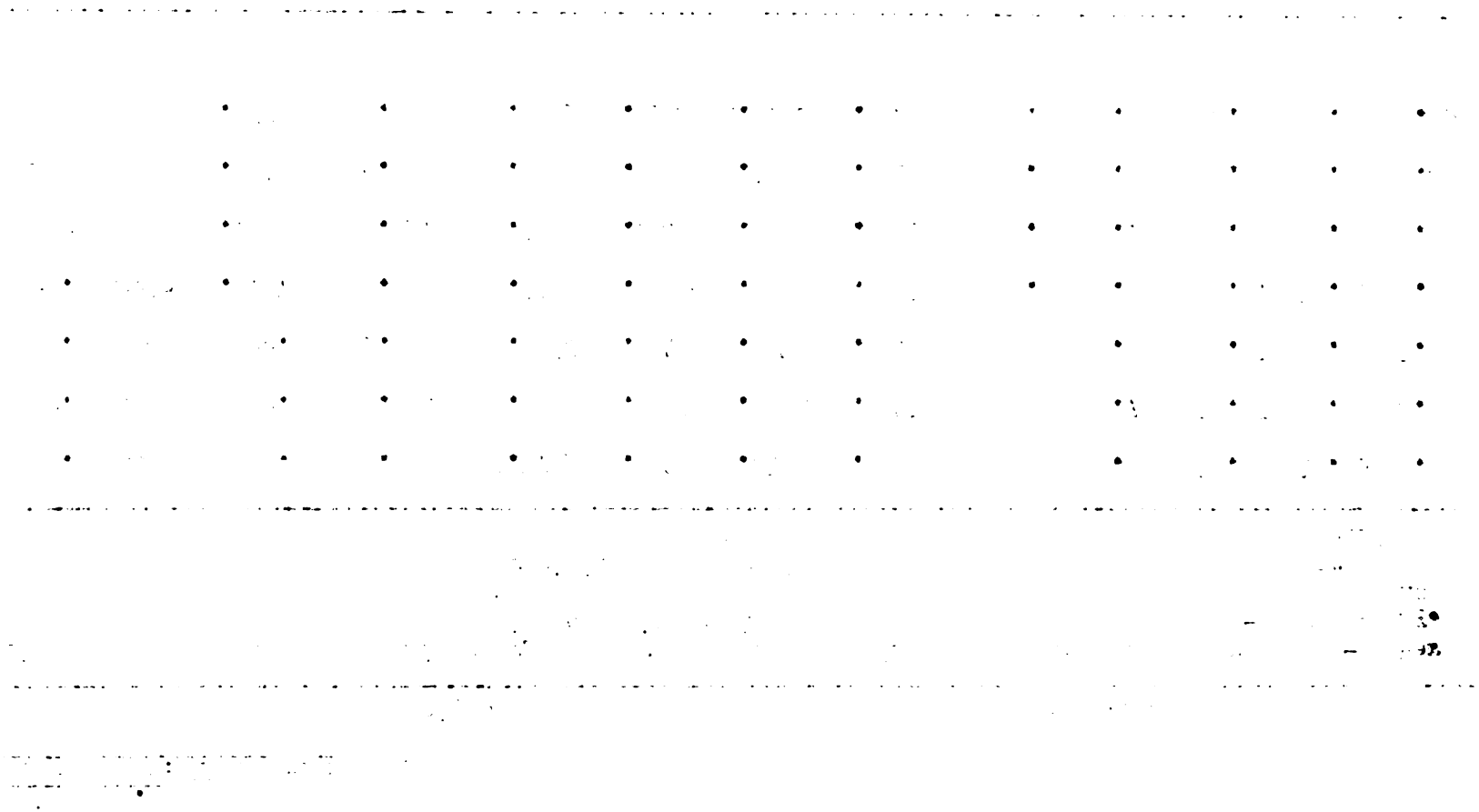


CALCIUM AND HISTAMINE

Animal No.3
Body Weight: 1100 Grams

	Weights						Percentage		Counts		
Tissue	Wet	Crucible	Dry Tissue and Crucible	Dry Tissue	Ash Tissue and Crucible	Ash	Water	Ash	Per Second	Corrected Counts Per Second	Per Mg. Ash
2 Hr. blood	1 cc.	4.6098	4.7723	.1625	4.6170	.0072		4.4	10.69	10.69	1.48
4 Hr. blood	1 cc.	6.0923	6.2862	.1939	6.0994	.0071		3.7	14.85	14.85	2.09
6 Hr. blood	1 cc.	5.4696	5.7068	.2372	5.4772	.0076		3.2	11.51	11.51	1.51
Pect. Muscle	.6787	5.9909	6.1455	.1546	6.0000	.0091	77.2	5.8	10.77	10.77	1.18
Liver	.7073	5.9378	6.1051	.1673	5.9483	.0105	76.3	6.3	8.00	8.00	.76
Lung	.3945	6.1433	6.2222	.0789	6.1489	.0056	80.0	7.1	7.22	7.22	1.29
Bladder	.4579	4.5675	4.6023	.0348	4.5700	.0025	92.4	7.2	2.63	2.63	1.05

All counts are corrected for background

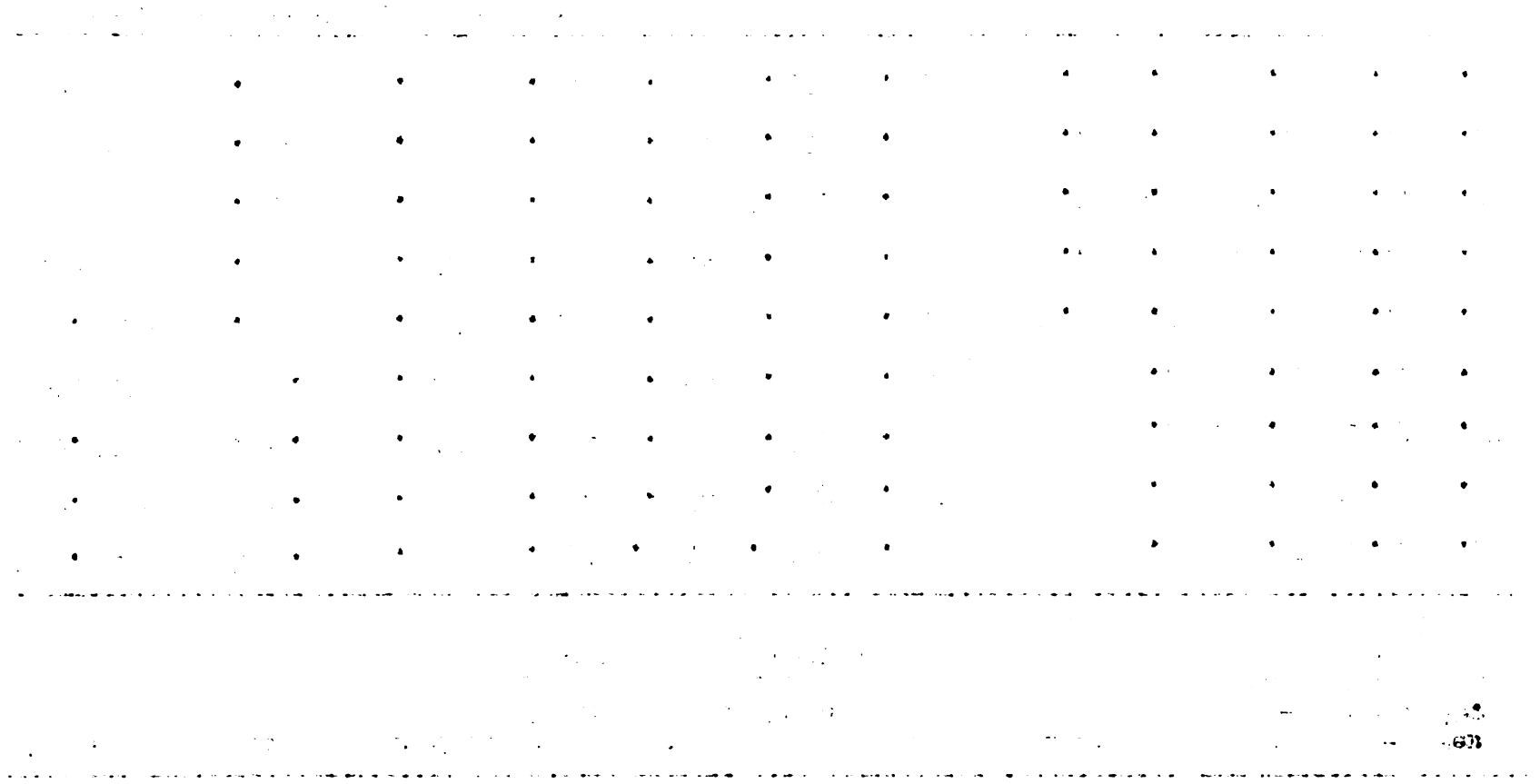


CALCIUM AND HISTAMINE

Animal No.6
Body Weight: 968 Grams

		Weights					Percentage		Counts		
Tissue	Wet	Crucible	Dry Tissue and Crucible	Dry Tissue	Ash Tissue and Crucible	Ash	Water	Ash	Per Second	Corrected Counts Per Second	Per Mg. Ash
2 Hr. blood	1 cc.	6.2140	6.4034	.1897	6.2226	.0086		4.5	13.61	13.61	1.58
4 Hr. blood	1 cc.	5.8482	6.0224	.1742	5.8563	.0081		4.6	15.50	15.50	1.91
6 Hr. blood	1 cc.	5.9070	6.0822	.1752	5.9153	.0083		4.7	12.23	12.23	1.47
Portal blood	½ cc.	4.6637	4.6733	.0096	4.6645	.0008		8.7	1.63	1.63	.20
Pect. Muscle	.4414	5.5984	5.6936	.0952	5.6036	.0052	78.4	5.5	29.78	29.78	5.73
Liver	.9074	5.5212	5.7508	.2296	5.5333	.0121	73.7	5.3	27.08	27.08	2.24
Lung	.2437	4.4955	4.5387	.0432	4.4985	.0030	82.3	6.9	4.17	4.17	1.39
Bladder	.1628	5.8796	5.9045	.0249	5.8816	.0020	84.7	8.0	5.79	5.79	2.90
Uterus	.3553	5.4271	5.4874	.0603	5.4310	.0039	83.0	6.5	6.62	6.62	1.70

All counts are corrected for background



CALCIUM AND HISTIDINE

Animal No.5
Body Weight: 990 Grams

		Weights					Percentage		Counts		
Tissue	Wet	Crucible	Dry Tissue and Crucible	Dry Tissue	Ash Tissue and Crucible	Ash	Water	Ash	Per Second	Corrected Counts Per Second	Per Mg. Ash
2 Hr. blood	1 cc.	5.6411	5.8746	.2335	5.6496	.0085		3.6	7.80	7.80	.92
4 Hr. blood	1 cc.	5.6028	5.7853	.1825	5.6091	.0063		3.5	8.77	8.77	1.39
6 Hr. blood	1 cc.								4.15	4.15	
Pect. Muscle	.4598	5.7003	5.7904	.0901	5.7056	.0053	80.5	5.9	8.31	8.31	1.57
Liver	.2616	6.0432	6.2337	.1905	6.0540	.0108	27.2	5.6	4.15	4.15	.38
Lung	.4493	5.9682	6.0446	.0764	5.9735	.0053	83.0	7.0	4.51	4.51	.85
Bladder	.3500	4.7503	4.7965	.0462	4.7512	.0009	86.8	1.9	4.43	4.43	4.92

All counts are corrected for background