

Report on Bromide Analyses on Urine from
Control Rabbits and from Rabbits exposed to 60 p.p.m.
Methyl Bromide

SUMMARY

Rabbits paralyzed by exposure to 60 p.p.m. methyl bromide show a four to five-fold increase in the bromide excreted per day corresponding to about a ten-fold increase in bromide excreted per cc. of urine. Rabbits begin to show this increased bromide excretion after the first exposure, this excretion increasing with each exposure until it very soon reaches that found in the paralyzed animals.

DATA AND DISCUSSION

Controls

The metabolism data for the three control rabbits is given in Table I and summarized in Table II. It will be seen that the bromide excreted per day per kilogram body weight averages 1.1 mg. (0.9 to 1.2 mg) and the bromide per cc. of urine averages 0.015 mg. (0.011 to 0.019 mg.). As a matter of record the food and water intake are given.

Rabbits exposed to methyl bromide

A. Paralyzed rabbits

The metabolism data on rabbits exposed to 60 p.p.m. methyl bromide are given in Table III and summarized in Table IV. Rabbit 5-218 was placed in the metabolism cage on the day of its 14th exposure when it was showing a definite, though slight, paralysis and daily urine specimens were taken until the time of death (after the 24th exposure). It will be noticed that the food and water intake of this animal compared favorably with that of the controls until paralysis became very marked and the animal was practically

side per kilogram per day, this increase was from 1.1 to 3.5, and as mg. bromide per cc. of urine it was from 0.015 to 0.115.

Similarly, in the case of rabbit 5-219, whose urine was collected from the 20th to 25th exposures, the bromide content of the urine was greatly increased. Expressed as mg. bromide per kilogram per day this increase was from 1.1 to 4.4 and as mg. per cc. of urine it was from 0.015 to 0.111. Neither the food nor water consumption of this animal was greatly diminished until the paralysis was markedly advanced and the rabbit was completely helpless. It will be seen that when a rabbit has been exposed to methyl bromide until paralyzed, the daily excretion of bromide (per kilogram body weight) is 4 to 5 times the normal excretion, while the excretion per cc. of urine is increased almost ten-fold.

B. Rabbits receiving their first exposures.

Since rabbits paralyzed by exposures to methyl bromide showed a great increase in urinary bromide, it was deemed advisable to study the bromide in the urine of rabbits during their first few exposures. The metabolism data on these animals is given in Table V and summarized in Tables II and IV. Each rabbit (R-5-277 and R-5-278) was placed in the metabolism cage and a 2-day composite of urine collected, after which daily specimens were collected during the first five exposures to 60 p.p.m. methyl bromide. Both rabbits appeared normal throughout the experiment. These animals were more nervous than the others used and did not become accustomed to the routine of the experiment. This nervousness probably accounts for the increased urine volume (particularly of R-5-277) and the more erratic consumption of food and water. However, both animals showed a substantial increase in bromide excretion after their first exposure, which increased with each additional exposure. This is shown nicely by a study of the last two columns of Table V (particularly mg. bromide per cc. of urine which increases from 0.029, the control value, to 0.035, 0.050, 0.068 on the first three days of

~~THE EFFECT OF METHYL BROMIDE ON THE URINARY EXCRETION OF BROMIDE IN RABBITS~~
bromide/mg./day as compared with 5.0 mg. bromide/kg./day for the paralyzed animals). The mg. bromide per cc. of urine for the first five day period is about half that of the paralyzed animals, a three to five-fold increase over the controls. Thus it is evident that the bromide excretion is increased almost immediately upon exposure to methyl bromide, this increased excretion becoming greater on each exposure, very rapidly approaching that found in the paralyzed animals. In other words, the high bromide excretion is present very much sooner than is the paralysis in rabbits exposed to 60 p.p.m. methyl bromide.

METHOD

Collection of the urine

The rabbit was placed in a monel metal metabolism cage of such construction that the urine was collected practically quantitatively, free from feces, food or other contaminating material. A daily record was kept of the food and water intake, the urine volume and the body weight of the animal. During exposures, the entire metabolism cage, with the exception of the containers for food and water, was placed within the fume chamber. The urine was preserved with chloroform and was usually analyzed within 48 hours after collection.

Bromide analysis of the urine

Bromide was determined by the method of Yates (Biochem. J. 27, 1763 (1933)).

An aliquot of the urine (3-5cc.) was heated with KOH, evaporated to dryness and heated at 500°C. for two hours in a nickel crucible. The extract was transferred to an aeration apparatus similar to that of Binnington (Ind. Eng. Chem. 29, 353 (1937)). The extract was acidified and the bromide oxidized to bromine by a chromic-sulfuric acid mixture. This was in such concentration that, while chloride remained unoxidized, bromide oxidation was complete. Bromine was removed by aeration and absorbed in starch iodide solution. The iodine liberated was then titrated with N/1000 thiosulfate.

Table I

Metabolism Data on Control Rabbits

Rabbit Number	Weight	Food	Water	Sample Number	Urine Volume	Sample Volume	Composite	Composite Volume	Aliquot	
	gm.	gm.	gm.		actual cc.	cc.		cc.	cc.	
R-5-89	3000	125	278	4	198	250	B	500	5	
	2850	107	265	5	196	250				
	2835	105	265	6	110	250	C	750	5	
	2910	120	295	7	170	250				
	2850	77	260	8	198	250				
	2820	105	279	9	138	250	D	750	5	
	2890	120	245	10	190	250				
	2860	95	230	11	142	250				
	2865	145	280	12	174	250	12	250	5	
	R-5-280	1698	80	180	1	130	130	A	300	5
		1695	25	85	2	150	170			
		1790	125	240	3	130	130	B	270	5
1790		115	210	4	140	140				
R-5-281	1520	80	140	1	98	100	A	250	5	
	1520	175	125	2	140	150				
	1610	90	200	3	122	122	E	292	5	
	1640	85	175	4	170	170				

Summary of Data on Urine from the Control Rabbits

Rabbit Number	Average Weight	Food/Day	Food/kg/Day	Water/Day	Water/kg/Day	Urine/Day	Urine/kg/Day	Br/Day	Br/kg/Day	Br/gg Urine
	gm	gm	gm	cc	cc	cc	cc	mg	mg	mg
R-5-89	2876	111	39	266	93	168	58	3.3	1.1	0.91
R-5-280	1763	86	49	179	101	137	77	1.5	0.9	0.91
R-5-281	1570	108	69	160	102	133	85	1.9	1.2	0.91
Average			52		99		73		1.1	0.91
R-5-277	1830					210	115	6.1	3.3	0.91
R-5-278	1605					103	64	2.8	1.7	0.91

Table III

Metabolism Data on Rabbits Exposed to 60 p.p.m. Methyl Bromide.

Rabbit Number	Weight	Food	Water	Sample Number	Urine Volume	Sample Volume	Composite	Compos. Volume	Aliquot	Tri- N/100
	gm	gm	gm		cc	cc		cc	cc	cc
R-218 14-24 exposure From time animal began to show paralysis until moritund	2068	70	160	1	146	250	A	750	5	3.04
	2060	70	185	2	50	250				
	2150	60	180	3	210	250				
	2050	65	171	4	86	250	B	750	5	3.04
	2089			5	118	250				
	2090	45	150	6	148	250				
	2055	105	210	7	124	250	C	750	5	3.04
	2085	45	155	8	140	250				
	2040	70	185	9	88	250				
	2070	40	100	10	54	100	D	250	5	4.1
	2060	115	145	11	125	150				
	1800	5	20	12	0		14	100	5	3.04
	1885	5	15	13	0					
	1885	0	10	14	48	100				
Dead - Urine from Bladder	1780			16	110	150				
R-5-219 20-25 exp. From time of fairly marked par- alysis until death Killed- Urine from Bladder	2000	65	120	1	168	250	A	500	5	3.04
	1895		155	2	66	250				
	1925	45	90	3	62	125	B	250	5	2.9
	1910	120	185	4	94	125				
	1750	0	15	5	50	50	C	200	5	3.04
	1760	0	15	6	0	0				
	1755	10	40	7	126	150				
	1805	0	0	8	36	50	D	100	5	2.7
	1860	-	-	9	36	50				

Rabbit	Condition	Average Weight	Urine per Day	Urine/kg/Day	Ue per Day	Ue/kg/Day	Ue/cc Urine
R-5-218	14-24th exposure Onset of paralysis to death	2009	98	49	11.1	5.5	0.115
R-5-219	20-25 exposure Fairly marked paralysis to death	1791	71	40	7.9	4.4	0.111
Average of paralyzed animals				45		5.0	0.113
R-5-277	Apparently normal First 5 exposures	1831	166	91	9.4	5.1	0.057
R-5-278	Apparently normal First 5 exposures	1551	99	64	4.3	2.8	0.043

Table V

Metabolism Data on Rabbits, Before Exposure and During Their First Five

Rabbit Number	Weight	Food	Water	Sample Number	Urine Volume	Sample Volume	Compos.	Compos. Volume	Aliquot	Titration/100 Thio
R-5-277	gm	gm	cc		cc	cc		cc	cc	cc
Control	1935	90	315	1	170	170	A	420	3	1.00
	1820	85	305	2	250	250				
Apparently normal First five exposures	1810	65	220	3	150	150	3	150	3	1.00
	1825	70	220	4	172	172	4	172	3	1.00
	1820	60	205	5	136	136	5	136	3	2.00
	1865	90	265	6	204	204	6	204	3	2.00
	1855	75	235	7	170	170	7	170	3	2.00
R-5-278										
Control	1600	60	165	1	100	100	A	206	3	0.00
	1610	65	140	2	106	106				
Apparently normal First 5 exposures	1610	50	110	3	98	98	3	98.3	3	1.00
	1565	60	140	4	90	90	4	90	3	1.00
	1565	25	95	5	80	80	5	80	3	2.00
	1550	10	110	6	92	92	6	92	3	1.00
	1495	0	100	7	136	136	7	136	3	1.00

Brom methane was sprayed intermittently for five minutes on the shaven abdomen of rabbit #2-156. A mist of fine drops came into contact with the skin and immediately vaporized. The skin was chilled to a considerable extent.

After 24 hours there was a definite redness of the treated area and a marked edema. This reaction disappeared within the following 24 hours, the skin appearing normal 48 hours after the treatment.

Application of Brom methane would appear to be followed by a marked local reaction characterized by redness and edema. This reaction is more severe than would be expected from the cooling effect alone. There was apparently no lasting damage or systemic reaction.

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