XC. THE NITRATE CONTENT OF ANIMAL TISSUES, AND THE FATE OF INGESTED NITRATE.

By MARY WHELAN.

From the Department of Biochemistry and Pharmacology School of Medicine, University of Oklahoma.

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PREVIOUS work with ammonium nitrate has shown that it is not completely excreted. In man approximately 80 % is excreted by the kidney during the period of intake. The total excretion may reach 90 % since nitrate continues to be excreted for several days after ingestion is discontinued. In dogs the percentage is not nearly so high. On an average, about 70 % is excreted by the kidney. The work of Keith *et al.* [1930] has shown that the amount of nitrate excreted by man in the faeces is negligible. That this is true in the case of dogs has not been proved. In future work on this problem we shall check that point. The fate of nitrate retained in the body is unknown. Nitrate taken by mouth appears quite soon in the blood stream, and analyses of oedema [Keith *et al.* 1930] and ascitic fluids show that it appears in these fluids in concentrations closely approaching that of the serum. Because of the ease with which it diffuses it comes in contact with all tissues. In an effort to determine what becomes of the nitrate retained this study was undertaken.

Method of study. Four dogs six weeks old and of the same litter were used. They were placed on a diet of 250 g. of prepared dog food per day. This was supplemented by a pint of milk and 500 ml. of water. Analysis showed that the nitrate content of food, milk and water was not appreciable. That this was an adequate diet is shown by the fact that the dogs gained weight steadily, on an average 1·7 kg. per dog during the 23 days of the experiment. The dogs receiving no nitrate gained 2 kg. each; the two receiving ammonium nitrate gained 1·4 kg. each. The exact significance of the difference is not apparent but it may be due to dehydration resulting from ingestion of such a large quantity of ammonium nitrate.

The dogs were kept on this diet for 7 days as a control period, and then dogs 1 and 2 were given 10 ml. of 10 % ammonium nitrate per day, by stomach tube, for 16 days. On the twenty-fourth day, 24 hours after the last dose of nitrate, the dogs were killed. This was done by inserting a cannula into the femoral artery, under local anaesthesia, and producing death by bleeding. This removed practically all the blood, as shown by the fact that little bleeding occurred when the tissues were removed. As soon as death had occurred the tissues and organs desired for analysis were removed and weighed. Each organ was then cut into small pieces and mixed, and representative samples were selected for determination of water and chloride contents. The remainder of the tissue was treated with sufficient sodium hydroxide to ensure an alkaline reaction throughout the drying process, and was then placed in a drying oven at 100° until thoroughly dry. It was then ground in a food chopper with a fine knife and was ready for analysis. The nitrate content was determined according to the technique outlined elsewhere [1935]. Chloride content was determined by the method of Wilson and Ball [1928]; the water content, by drying to constant weight. All determinations were made in duplicate and some of the nitrate determinations were made in triplicate. Nitrate in blood and urine was determined according to the method of Whelan [1930].

During the entire period of the experiment, the dogs were kept in metabolism cages and the urine collected under toluene. On each twenty-four hour specimen, volume, p_H and nitrate-nitrogen were determined (Table II). Blood was analysed at intervals for chloride and nitrate-nitrogen (Table I).

Table I. *Chloride and nitrate-nitrogen content of blood.*

mg./100 ml.

	Dog 1		Dog 2		Dog 3		Dog 4	
Date	Cl	NO_3-N	Cl	NO_3-N	Cl	NO_3-N	Cl	NO_3-N
19. iii	361	0	396	0	410	0	370	0
26. iii	386	5·3	390	5·5	—	—	—	—
4. iv	408	6·2	408	5·2	420	0	400	0

Table II. *Chloride and nitrate-nitrogen of urine.*

	Urine		Chlorine		Nitrate-N		
Date	Average volume	p_H range	g./100 ml. average	Total g. average	g./100 ml. average	Total g. average	Dog
12–19. iii	184·4	5·8–6·5	0·408	0·7504	0	0	1
19. iii–4. iv	319·7	6·5–5·2	0·350	1·211	0·0305	0·091	1
12–19. iii	95	5·8–6·5	0·485	0·459	0	0	2
19. iii–4. iv	239·4	6·5–5·2	0·348	0·835	0·0322	0·077	2
12–19. iii	139	5·7–6·5	0·509	0·650	0	0	3
19. iii–4. iv	340	6·3–6·9	0·379	1·29	0	0	3
12–19. iii	348	6·0–6·9	0·358	1·245	0	0	4
19. iii–4. iv	264	6·3–6·2	0·363	0·96	0	0	4

Discussion. As has been repeatedly observed, the ingestion of ammonium nitrate increased the average daily excretion of urine and decreased the p_H. The urine remained negative for nitrate during the control periods, and in the case of the dogs receiving no nitrate remained nitrate-free during the entire period of the experiment. The average daily excretion of nitrate-nitrogen during the period of ingestion in dogs 1 and 2 was 0·0305 and 0·0322 g. respectively. Although the concentrations were similar, less total nitrate was excreted by dog 2 than by dog 1. Dog 1 excreted 53·8 % of that ingested and dog 2, 45·4 %. At the time of death approximately half of the nitrate had not been excreted. The possibilities are: (1) that it was all retained and had been taken up by the various tissues, possibly replacing chloride; (2) that it had been destroyed, by being broken down into some form which does not produce a colour reaction with diphenylbenzidine; (3) that it entered into organic combination of some type, which is not altered by treatment with acid, and therefore cannot be extracted with ether; (4) that it is not completely absorbed from the gastro-intestinal tract. Possibilities (2) and (3), as will be shown later, are substantiated by data.

Analyses of tissues.

Chloride. Chloride determinations (Table III) were made on all tissues. The results were variable and seemed to bear no relation to nitrate ingestion. The table is included because the variability of results may have some bearing on the analysis of the problem.

Nitrate. Table IV gives the results of analyses of tissues for nitrate-nitrogen. In all cases, with the exception of the oesophagus, the nitrate contents of the tissues of dogs 1 and 2 were markedly higher than those of the corresponding tissues of

Table III. *Chloride content of tissues.*

Chloride g./100 g.

Tissue	Dog 1	Dog 2	Dog 3	Dog 4
Pancreas	0·137	0·172	0·153	0·168
Heart	0·128	0·143	0·128	0·103
Kidneys	0·257	0·246	0·256	0·242
Spleen	0·172	0·149	0·168	0·157
Trachea	0·179	0·178	0·226	0·185
Oesophagus	0·135	—	0·126	—
Bladder	0·191	0·149	0·216	0·197
Brain	0·162	0·171	0·164	0·153
Spinal cord	0·153	0·179	0·199	0·186
Lung	0·231	0·217	0·186	0·192
Liver	0·131	0·106	0·122	0·102
Stomach	0·123	0·193	0·173	0·108
Intestine	0·090	0·126	0·101	0·084
Muscle	0·113	0·108	0·120	0·128
Skin	0·211	0·212	—	0·184

Table IV. *Nitrate-nitrogen content of tissues.*

Nitrate-N mg. per 100 g. dry tissue.

	Received nitrate						
Tissue	Dog 1	Dog 2	Dog 3 Control	Dog 4 Control	Average 1–2	Average 3–4	Average difference
Brain	1·03	1·00	0·60	0·57	1·02	0·59	0·43
Spinal cord	1·39	1·30	0·96	0·86	1·35	0·91	0·44
Trachea	1·86	1·51	0·50	0·43	1·69	0·47	1·22
Lung	0·97	1·20	0·53	0·73	1·09	0·63	0·40
Heart	1·35	1·39	0·82	0·81	1·38	0·82	0·46
Stomach	1·15	0·85	0·37	0·40	1·00	0·53	0·47
Pancreas	1·58	1·64	0·61	0·53	1·61	0·57	1·04
Liver	1·43	1·50	0·65	0·64	1·47	0·65	0·82
Spleen	1·96	1·50	0·40	0·60	1·73	0·50	1·23
Kidney	1·15	1·46	0·67	0·77	1·32	0·72	0·60
Intestine	1·00	0·70	0·63	0·32	0·85	0·48	0·37
Muscle	1·86	1·40	0·84	0·74	1·63	0·84	0·79
Oesophagus	1·24	—	1·21	—	1·24	1·21	0·03
Skin	1·46	1·11	0·78	0·95	1·29	0·87	0·42
Bladder (urinary)	1·17	1·28	1·00	—	1·23	1·00	0·23

dogs 3 and 4. Taking the average difference between the tissues of dogs which had received nitrate and of those which had received none shows that the greatest difference occurs in trachea, pancreas, liver, spleen, kidney and muscle. Of these, the largest differences occurred in trachea, pancreas and liver; the least differences occurred in the oesophagus; the urinary bladder and intestine also showed very small differences. In the other tissues, namely brain, spinal cord, lung, heart, stomach and skin, the difference in nitrate content between control and nitrate-fed dogs is marked and almost identical in all cases.

Table V gives the weights of fresh tissues, the percentage of water in each, and the calculated concentration of nitrate-nitrogen in the wet tissues as well as the total nitrate-nitrogen content of each. It emphasises the facts brought out

Table V. *Nitrate-nitrogen content of wet tissue.*

	Dog 1	Dog 1 Nitrate-N			Dog 2	Dog 2 Nitrate-N			Dog 3	Dog 3 Nitrate-N			Dog 4	Dog 4 Nitrate-N		
Tissue	wt. g.	mg./100 g.	mg. total	H_2O %	wt. g.	mg./100 g.	mg. total	H_2O %	wt. g.	mg./100 g.	mg. total	H_2O %	wt. g.	mg./100 g.	mg. total	H_2O %
Brain	63·2	0·176	0·112	82·8	60·88	0·186	0·113	81·4	72·89	0·106	0·077	82·3	75·87	0·105	0·080	81·6
Spinal cord	10·7	0·317	0·034	77·2	10·38	0·385	0·037	72·4	11·37	0·263	0·030	72·6	9·68	0·240	0·022	72·1
Trachea	15·21	0·586	0·090	68·4	11·46	0·384	0·044	74·6	15·67	0·113	0·017	77·7	14·29	0·103	0·015	76·0
Lungs	47·53	0·201	0·096	79·3	57·79	0·242	0·140	79·8	53·39	0·110	0·058	79·2	54·79	0·158	0·087	78·3
Heart	34·58	0·230	0·090	79·4	35·62	0·295	0·105	78·8	32·08	0·161	0·052	80·3	33·85	0·169	0·057	79·4
Stomach	60·90	0·214	0·131	81·35	50·12	0·173	0·087	79·6	60·73	0·083	0·051	77·6	75·13	0·063	0·047	84·3
Pancreas	14·93	0·296	0·044	81·3	11·5	0·323	0·037	80·3	13·53	0·147	0·020	75·9	13·97	0·142	0·020	73·3
Liver	218·43	0·343	0·750	76·0	197·18	0·402	0·770	73·2	224·62	0·184	0·413	71·7	241·00	0·154	0·372	71·0
Spleen	8·94	0·406	0·036	79·3	11·34	0·316	0·036	79·0	8·015	0·074	0·006	81·5	10·49	0·127	0·013	78·8
Kidneys	38·92	0·230	0·090	77·0	34·37	0·314	0·108	78·5	42·48	0·138	0·059	79·4	41·66	0·172	0·072	77·7
Intestine	301·2	0·166	0·500	83·45	253·88	0·155	0·392	77·8	312·2	0·117	0·365	81·35	356·9	0·060	0·215	81·2
Muscle	2482·0	0·512	12·708	72·5	2230·0	0·356	7·950	74·6	2570·0	0·211	4·94	74·8	2660·0	0·206	5·500	72·3
Oesophagus	15·62	0·279	0·044	77·5	—	—	—	—	20·31	0·235	0·048	80·6	—	—	—	—
Skin	950·0	0·806	7·660	44·7	840·0	0·534	4·480	52·0	968·0	—	—	—	998·2	0·60	5·980	37·0
Bladder	3·5	0·377	0·0132	67·8	3·25	0·400	0·013	68·7	5·34	0·231	0·012	76·9	4·18	—	—	72·7
Blood	406·0	6·2	25·200	—	364·0	5·2	18·90	—	420·0	—	—	—	434·0	—	—	—
Dog	5800·0	—	—	—	5200·0	—	—	—	6000·0	—	—	—	6200·0	—	—	—

in Table IV, but the values shift somewhat because of differences in water content. In this table are included the estimated total contents of muscle, skin and blood. The calculations were made by using the figures given by Skelton [1927] for the proportion of the weight of the organs to the total body weight.

Table VI gives the total values for nitrate-nitrogen ingested, excreted and retained, as well as total amounts determined in the tissues. The nitrate content of tissues of the dogs which received nitrate was six to eight times higher than

Table VI. *Relationship between nitrate-nitrogen ingested, excreted and retained.*

	Dog 1 mg.	Dog 2 mg.	Dog 3 mg.	Dog 4 mg.
Nitrate-N ingested	2700	2700	0	0
Nitrate-N excreted	1452	1225	0	0
Nitrate-N retained	1248	1475	0	0
Nitrate-N in tissues	47·59	33·21	6·15	10·18
Nitrate-N in tissues per kg. body weight	8·21	6·39	1·02	1·66

that of those which did not receive nitrate. Dog 2 excreted a smaller percentage of nitrate than dog 1, and the nitrate content of the total tissues was also smaller. But the differences are slight in comparison to the total amount of nitrate retained.

This shows, then, that nitrate retained in the body is either destroyed or enters into some organic combination which is not broken down by the treatment to which we have subjected these tissues. Upon this theory we are undertaking some further work on this problem. There is also the possibility that nitrate taken by mouth by dogs is not completely absorbed from the gastro-intestinal tract. From experimental work on human subjects, it would seem that this is quite unlikely. However, the possibility of this fact accounting for the loss of 50 % of the total amount seems to be very remote. As shown in Table VI, the amount of nitrate which can be accounted for in the tissues is less than 4 % of the difference between the amount ingested and that excreted in the urine. This leads us to the conclusion that nitrate is destroyed or altered by the tissues and therefore accounts for the fact that it is not recovered quantitatively in the urine.

Table VII shows that only 75 % of the nitrate-nitrogen added to the dry tissue *in vitro* can be recovered. But pure potassium nitrate added to the

Table VII. *The recovery of nitrate added to tissues.*

Weight of sample g.	NO_3-N in sample mg.	NO_3-N added mg.	NO_3-N determined mg.	NO_3-N recovered mg.	% recovered
2	0·0275	0·05	0·055	0·0375	75
2	0·0169	0·05	0·057	0·0386	77·5

same amount of asbestos fibre as is used in all extractions can be completely recovered.

Summary.

1. The nitrate-nitrogen content of animal (dog) tissues is given. Tissues analysed were taken from dogs which had received no additional nitrate, as well as from dogs which had received large amounts of ammonium nitrate. The latter showed an increase of six to ten times the content of normal tissues.

2. Approximately 50 % of the nitrate-nitrogen ingested was excreted by the kidney.

3. Although it has not been shown experimentally in dogs, it seems unlikely that lack of absorption from the gastro-intestinal tract explains the recovery of only 50 % in the urine.

4. Analyses showed that only a small part is taken up by the tissues and retained as inorganic nitrate.

5. The findings in this study suggest that nitrate is destroyed in the tissues and this explains why it cannot be recovered quantitatively in the urine.

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